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Research Article

Detection and expression analysis of recombinant proteins in plant-derived complex mixtures using nanoUPLC-MS^E

The use of mass spectrometry to identify recombinant proteins that are expressed in total soluble proteins (TSPs) from plant extracts is necessary to accelerate further processing steps. For example, the method consists of TSP sample preparation and trypsin digestion prior to the preliminary characterization using nanoUPLC-MS^E analysis of the recombinant proteins that are expressed in TSP samples of transgenic soybean seeds. A TSP sample as small as 50 µg can be effectively analyzed. In this study, transgenic soybean seeds that expressed recombinant cancer testis antigen (CTAG) were used. The procedure covered 30% of the protein sequence and was quantified at 0.26 ng, which corresponded to 0.1% of the TSP sample. A comparative proteomic profile was generated by the comparison of a negative control and sample that showed a unique expression pattern of CTAG in a transgenic line. The experimental data from the TSP extraction, sample preparation and data analysis are discussed herein.

Keywords: Expression^E / Identity^E / NanoLC-MS^E / ProteinLynx global server / Soybean

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1 Introduction

The production of recombinant proteins is an important step in several academic, industrial and pharmaceutical processes. Several heterologous protein expression systems are available, including bacterial [1], mammalian cell culture [2] and plant [3, 4] systems. Although these systems constitute the main production systems, the search for novel methods to increase protein yield, facilitate manipulation and reduce cost is ongoing. Seeds are an advantageous production platform for recombinant proteins for several reasons: they can undergo long-term storage at ambient temperatures [5, 6], they can provide an appropriate biochemical environment for protein stability through the creation of specialized storage compartments [6, 7], they are not contaminated by human or animal pathogens [8], their desiccation characteristics prevent them from undergoing non-enzymatic hydrolysis or protease degradation [5, 8], and they do not carry the harmful substances that are present in some plant leaves, which is important for downstream

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Abbreviations: CTAG, cancer testis antigen 1; PLGS, Protein-Lynx global server; TSP, total soluble protein processing [3, 8]. We recently produced several transgenic soybean plants that express important pharmaceutical molecules, such as proinsulin [6], human growth hormone (hGH) [9] and human coagulation factor IX (hFIX) [10], which demonstrate the versatility of this system. However, the production of these transgenic lines is extremely time consuming [11] and requires at least 150 days to obtain the first seeds and another three years to generate a homozygous line. During the early stages, there is little material available for recombinant protein purification. As a result, the detection, quantification and characterization of recombinant molecules rely mainly on the manipulation of the total soluble protein (TSP), which is a complex mixture that contains a low abundance of the protein of interest. Therefore, a method that detects, qualifies and quantifies recombinant proteins in TSP using < 1/4 of a single seed mass (\approx 50 mg) is needed.

Typically, a recombinant protein is identified using Western blot analysis [12] and quantified using enzymelinked immunosorbent assays (ELISAs) [13]. These methods are widely used because they are simple and relatively fast for identification and quantification, but they lack sensitive detection ability when small amounts of antigens are used, no antibody is available, or a false positive is found, and there is no way to verify the quality, amino acid sequence or post-translational modifications of the recombinant protein. Two dimensional electrophoresis (2-DE) was developed for proteomics [14, 15], and because of its association with MS, it has become the primary tool for the identification and characterization of complex plant mixtures [15, 16]. 2-DE can also be used for the quantification and protein mapping of tissues [17], comparative proteomics [18, 19] and posttranslational modification identification [20]. However, this technique requires a minimum sample amount, cannot detect low-abundance molecules, requires spot manipulations for successful identification of proteins [15], is performed mainly using peptide mass fingerprinting (PMF) [21, 22], and has difficulty analyzing proteins with similar mass and pI because they appear as a single spot. The combination of gel and liquid chromatography mass spectrometry (LC-MS) methods may result in the better identification of proteins in complex samples [23, 24] and may overcome the drawbacks of 2-DE. LC increases the low detection/resolution of complex mixtures on mass spectrometers (MS) [25]. Furthermore, the analysis of peptides or complex samples that have been digested by trypsin, commonly known as "system samples", is key for the detection of low-abundance proteins, but this technique has limitations in analyte dilution, the minimum amounts of complex protein mixtures that are needed to guarantee a satisfactory dynamic range and the detection of low-abundance proteins [14, 15, 25, 26].

Nano-scale liquid chromatography combined with mass spectrometry with data-independent acquisitions (nanoLC-MS^E) has several benefits for proteome analysis. Among these benefits are detection and linear sequence structural information at the femtomole level [12, 27]; small surface areas and minimal dead volumes, which minimize analyte losses due to surface adsorption; and low flow rates, which reduce analyte dilution. Low-abundance analytes can be separated with a high recovery rate when they are associated with a high dynamic range and a high-quality MS detection system [13]. Recently, the nanoLC-MS method has been used for the detection of differences in the expression of soybean plasma membrane proteins under osmotic stress [28], the regulation of stress identification genes induced by iron deficiency in tomatoes [29] and the detection of neuropeptides that are secreted by Cancer borealis [30], which demonstrates the capability and potential of this method.

Moreover, nanoLC-MS is an important tool for the characterization of post-translational modifications of proteins, such as the identification of N-terminal peptide modifications in the chloroplast proteome [31], the analysis of human protein oxidations that lead to functional reduction/annulation [32], and the characterization of phosphorylation patterns of several phosphatase splice variants that are expressed in a human cell line [33, 34]. Finally, quantification is also possible with the nanoLC-MS technique

using labeling methods, such as the (18 O) labeling of peptides [35] and the iTRAQTM method [36], which are based on relative quantification methods, such as the use of stochastic measurements of mass and intensity deviations for each ion that is detected [37] or the absolute quantification based on a constant ion current that is acquired with low and high collision energies into the mass spectrometer, called MS^E [38–41]. MS^E is a data-independent acquisition method that uses low and high collision energies with no precursor selection, which is different from other methods, such as data-dependent acquisition (DDA). The ion detection, clustering and normalization of data-independent, alternate scanning LC-MS^E data have been explained in detail elsewhere [39].

Here, we describe an easy-to-handle, label-free nanoUPLC-MS^E method that provides absolute quantification and allows the use of small samples for the detection, quantification and characterization of low-abundance recombinant proteins that are expressed in soybean seeds, specifically the immunogenic tumor NY-ESO-1 antigen (cancer testis antigen 1, CTAG) [42]. CTAG is a protein product of a gene located on the human X chromosome that comprises 180 amino acid residues (Fig. 2) with a mass of 18 kDa, a glycine-rich N-terminal region and an extremely hydrophobic C-terminal region that is so insoluble that it bears striking resemblance to a transmembrane domain [42, 43]. Because of the insolubility of the C-terminal region, the production and subsequent identification and characterization of this protein from TSP extracts present a challenge. Expression pattern analysis using RT-PCR has confirmed that CTAG expression is restricted to the testis. It is not present in other normal tissues, but it is found in several types of cancer, including bladder, breast and lung cancers [44]. The recombinant CTAG that is produced in Escherichia coli (E. coli) was the first to be evaluated in the clinical setting in a study that ranks among the most promising CTAG trials that have been published because of the favorable and broadly applicable immunological clinical results [42, 45]. However, the use of CTAG as a vaccine is viable only if it is coupled with a low-cost, scalable recombinant protein production system.

2 Materials and methods

2.1 Chemicals and reagents

Water was purified using a Milli-Q purification system (Millipore, France) to obtain a conductivity of $< 1.3 \,\mu$ S/cm, a

MOAEGRGTGG STGDADGPGG PGIPDGPGGN AGGPGEAGAT 1 51 ARASGPGGGA PRGPHGGAAS GLNGCCRCGA RGPESRLLEF 101 EAELARRSLA QDAPPLPVPG VLLKEFTVSG NILTIRLTAA 151 SCLOOLSLLM WITOCFLPVF LAOPPSGORR

Matched to a peptide

Matched to a partial peptide

GGRGPRGAGA YLAMPFATPM DHRQLQLSIS

> **Figure 1.** The CTAG amino acid sequence. The colored boxes indicate the peptides found using PLGS (Table 2), and the overlapping regions are indicated by changes in color.



Figure 2. Experimental spectra results. (A) The nanoUPLC chromatogram of soybean CTAG lineage 3. The arrows indicate the eluted peptides corresponding to the CTAG-digested protein (Table 2). (B) MS spectra from the 110.31 min containing the $[M+2H]^{+2} = 857.87$ ion from the CTAG protein fragment. (C) Reconstructed product ion spectra from the $[M+2H]^{+2} = 857.87$ ion precursor. (D) Deconvoluted MS/ MS spectra processed using PLGS and the peptide sequence corresponding to ion [M+H] (1.1)⁺ = 1715.01 from the trypsin digestion of CTAG.

total organic carbon (TOC) $<2\,ppb$, and a semiconductor equivalent specification of $0.055\,\mu S/cm$ (18.2 m $\Omega\,cm$) at point-of-use at 25°C. Analytical-grade petroleum ether, 30–75°C, was purchased from J.T. Baker. Tris base

(2-amino-2-[hydroxymethyl]-1,3-propanediol), KCl, DL-dithiothreitol (threo-1,4-dimercapto-2,3-butanediol; DTT), phenylmethanesulfonyl fluoride (PMSF) \geq 98.5%, sodium dodecyl sulfate (SDS) for molecular biology \geq 98.5%, acetone



Figure 2. Continued.

CHROMASOLV[®] Plus \geq 99.9%, ammonium bicarbonate \geq 99.0%, iodoacetamide, trifluoroacetic acid, acetonitrile, formic acid and [Glu¹]-fibrinopeptide B, human (GFP) were purchased from Sigma-Aldrich. MassPREP Protein Digestion Standard Alcohol Dehydrogenase (ADH) and *Rapi*-GESTTM SF were purchased from Waters (Waters, USA). Sequencing-grade trypsin was purchased from Promega.

2.2 TSP extraction from recombinant CTAG soybean seeds

Two samples, Soy CTAG L3 and Soy Cn negative seeds from the BR-16 cultivar, were used in this work. The soybean seeds were ground into a fine powder in a coffee grinder. A 100 mg sample of powder was weighed and placed into a 2 mL capped centrifuge tube. Petroleum ether (1 mL) was added, and the sample was slowly agitated for 15 min. The supernatant was discarded, and this step was repeated twice $(2 \times)$. The petroleum ether was evaporated for 10 min, and 1 mL of 20 mM Tris-HCl, pH 8.3, 1.5 mM KCl, 10 mM DTT, 1 mM PMSF and 0.1% v/v SDS were added. The sample was slowly vortexed at room temperature for 10 min and centrifuged for 5 min at 5000 rpm at 4°C. The supernatant was then transferred to a new centrifuge tube. For each $200 \,\mu\text{L}$ of sample, $800 \,\mu\text{L}$ of cold acetone was added to the centrifuge tube. The sample was vortexed thoroughly and kept at -20° C for 1 h, with vortexing every 15 min. The sample was then centrifuged for 10 min at 13000 rpm. The supernatant was discarded, and the pellet was dried at room temperature for 30 min. The pellet was carefully dissolved in 500 µL of 50 mM NH4HCO3 and quantified using the Quant-iTTM Protein Assay Kit (Invitrogen, USA). The sample was finally diluted with 50 mM ammonium bicarbonate to a concentration of $1 \mu g/\mu L$.

2.3 Sample preparation for nanoUPLC-MS^E acquisition

An aliquot of 50 μ L of the 1 μ g/ μ L sample was added to 10 µL of 50 mM ammonium bicarbonate in a microcentrifuge tube. Next, $25 \,\mu L$ of $RapiGEST^{TM}$ (0.2% v/v) was added, and the sample was vortexed and incubated in a dry bath at 80°C for 15 min. The sample was briefly centrifuged, and $2.5\,\mu\text{L}$ of 100 mM DTT was added. The sample was vortexed gently and incubated at 60°C for 30 min, followed by centrifugation. Iodoacetamide $(2.5 \,\mu\text{L} \text{ of a } 300 \,\text{mM}$ solution) was added, and the sample was vortexed slightly and incubated in the dark at room temperature for 30 min. Next, $10 \,\mu\text{L}$ of trypsin (with $400 \,\mu\text{L}$ of $50 \,\text{mM}$ ammonium bicarbonate added per 20 µg vial of trypsin) was added, and the sample was vortexed slightly. The sample was digested at 37°C in a dry bath overnight. To precipitate the RapiGEST, 10 µL of a 5% TFA solution was added, and the sample was vortexed, incubated for 90 min at 37°C in a dry bath, and centrifuged at 14 000 rpm at 6° C for 30 min. The supernatant was transferred to a Waters Total Recovery vial (Waters), and 5 µL of ADH (with 1 mL of 3% acetonitrile and 0.1% formic acid) and 85 μ L of a 3% acetonitrile, 0.1% formic acid solution was added. The final concentration of the protein was 250 ng/ μL , and the final concentration of ADH was 25 fmol/ μL . The final volume was 200 μL .

2.4 NanoLC-MS^E acquisition

Nanoscale LC separation of tryptic peptides from TSP was performed using a nanoACQUITYTM system (Waters) equipped with a Symmetry C18 5 μ m, 5-mm \times 300- μ m precolumn and a nanoEaseTM BEH130 C18 $1.7 \mu m$, $100\,\mu m imes 100\,mm$ analytical reversed-phase column (Waters). The samples were initially transferred to the precolumn using an aqueous 0.1% formic acid solution with a flow rate of 15 µL/min for 1 min. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile. The peptides were separated using a gradient of 3-40% mobile phase B for 200 min with a flow rate of 600 nL/min followed by a 10 min rinse with 90% of mobile phase B. The column was re-equilibrated to the initial conditions for 20 min. The column temperature was maintained at 35°C. The lock mass was delivered from the auxiliary pump of the nanoACQUITY pump using a constant flow rate of 150 nL/min at a concentration of 100 fmol of GFP to the reference sprayer of the NanoLock-Spray source of the mass spectrometer. All samples were analyzed in triplicate.

The tryptic peptides were analyzed using a Synapt HDMSTM mass spectrometer (Waters, Manchester, UK) with a hybrid quadrupole/ion mobility/orthogonal acceleration time-of-flight (oa-TOF) geometry. For all measurements, the mass spectrometer was operated in the "Vmode" of analysis with a typical resolving power of at least 10 000 full-width half-maximum (FWHM). All analyses were performed using a positive nanoelectrospray ion mode (nanoESI⁺). The time-of-flight analyzer of the mass spectrometer was externally calibrated with GFP b+ and y+ ions from m/z 50 to 1990 with the data post-acquisition lock mass corrected using the GFP double charged precursor ion $[M+2H]^{2+} = 785.8426$. The reference sprayer was sampled at a frequency of 30 s. Exact mass retention time (EMRT) [46] nanoLC-MS^E data were collected in an alternating lowenergy and elevated energy mode of acquisition. The continuum spectra acquisition time, in each mode, was 1.5 s with a 0.1 s interscan delay. In the low-energy MS mode, data were collected at constant collision energy of 3 eV. In

Table 1. Peptide sequences found using PLGS for CTAG protein

Precursor MH+(Da)	Charge state	MH+Error (Da)	Score	Start	End	Sequence	Modifications	Retention time (min)	Intensity
1715.0154	2	0.0032	7.8859	108	124	(R)SLAQDAPPLPVPGVLLK(E)		110.31	19 656
1349.7391	2	0.0019	7.2048	125	136	(K)EFTVSGNILTIR(L)		94.51	7191
1871.1141	3	-0.0038	7.0925	107	124	(R)RSLAQDAPPLPVPGVLLK(E)		102.18	2493
2485.308	3	0.0177	6.1839	87	107	(R)LLEFYLAMPFATPMEAELARR(S)	Oxidation M (8)	141.81	2994
2855.4158	4	0.0052	6.0799	82	106	(R)GPESRLLEFYLAMPFATPMEAELAR(R)	Oxidation M (13)	63.48	2744

Table 2. Proteins identified using PLGS in the Soy CTAG L3 transgenic soybean line

Entry	Description	MW (Da)	р <i>I</i> (рН)	PLGS	Amount	% of TSP
				score	(ng)	
D702E0	Concertectio opticen 1	17 001	0 /7	2006 20	0.26	0.11
F70300 022120	a-Subunit of B-conducinin Fragment OS Glucine max PE 2 SV 2	63 126	0.47 4.72	2000.30	0.20	28.43
C6T488	Putative uncharacterized protein OS Glycine max PE 2 SV 1	24 103	5.13	49 332 05	0.47	0
P04776	Glycinin G1 OS Glycine max GN GY1 PE 1 SV 2	55 671	5.82	34 638.61	28.68	12.45
P19594	2S albumin OS Glycine max PE 1 SV 2	18 447	5.01	26 866.75	6.98	3.03
Q549Z4	Proglycinin A2B1 OS Glycine max PE 2 SV 1	54 356	5.29	26 163.09	9.12	3.96
P04405	Glycinin G2 OS Glycine max GN Gy2 PE 1 SV 2	54 356	5.29	26 155.1	16.83	7.31
C6TKH0	Putative uncharacterized protein OS Glycine max PE 2 SV 1	31 640	6.41	25 943.07	4.40	1.91
B3TDK4	Lipoxygenase OS Glycine max PE 3 SV 1	94 352	5.87	23 234.38	11.22	4.87
P08170	Seed lipoxygenase 1 OS Glycine max GN LOX1 1 PE 1 SV 2	94 310	5.93	22 866.77	0	0
P01063	Bowman Birk-type proteinase inhibitor C II OS Glycine max PE 1 SV 2	9194	4.37	19 673.12	2.77	1.20
P01064	Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2	9460	4.66	18 789.71	1.43	0.62
P24337	Hydrophobic seed protein OS Glycine max PE 1 SV 1	8353	6.04	17 254.79	0.54	0.23
039805	Dehydrin-like protein OS Glycine max PE 2 SV 1	23 703	6.084	16 428.98	5.44	2.36
07GC77	Glycinin A3B4 subunit OS Glycine max PE 1 SV 1	58 151	5.41	14 016.68	0.31	0.13
085204	Glycinin A1bB2 784 OS Glycine max PE 2 SV 1	54 264	5.94	12 395.54	0.65	0.28
U852U5	Glycinin A1bB2 445 US Glycine max PE 2 SV 1	54 183	5.//	12 393.59	0.49	0.21
P05046	Lectin US Glycine max GN LE1 PE 1 SV 1	30 908	5.59	11 981.52	8.64	3.75
061925	Putative uncharacterized protein US Glycine max PE 2 SV 1	42 /96	6.29	11 699.31	1.09	0.47
	Putative uncharacterized protein US Glycine max PE 2 SV 1	41 854	0.99	0064.62	0.50	0.22
UNCENO UNCENO	Seed maturation protein PM25 US Glycine max GN PM25 PE 2 SV 1	25/13	4.70	9904.0Z	1.03	0.40
USSEN0	Seed maturation protein PM20 US Glycine max GN PM20 PE 2 SV 1	20 087	4.03	9//0.58	0.97	0.42
	Seed maturation protein PN21 OS Chycine max CN PN21 PE 2 SV 1	17 730	0.10	9100.43	0.97	0.00
093EL0	Sood maturation protein PM22 OS Glycine max GN PM22 PE 2 SV 1	16 677	4.57	7063 37	0.67	0.30
	Sold maturation protein PM34 OS Glycine max GN PM34 PE 2 SV 1	21 7/6	4.30 6.68	7863 56	0.01	0.20
C6T107	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17 812	5.95	7729.99	1 31	0.10
C6T588	Putative uncharacterized protein OS Glycine max PE 2 SV 1	16 750	4 53	7076 10	0.60	0.37
09AVK8	Allergen Glv m Bd 28K Fragment OS Glvcine max PE 2 SV 1	52 608	5.65	5503.08	2.86	1.24
Q2I0H4	Glyceraldehyde 3 phosphate dehydrogenase OS Glycine max GN GAPC1 PE 2 SV 1	36 741	6.84	5307.64	1.46	0.63
Q9XET0	Putative uncharacterized protein OS Glycine max GN PM30 PE 2 SV 1	15 088	9.42	5145.05	0.76	0.33
C6TBB3	Putative uncharacterized protein OS Glycine max PE 4 SV 1	12 337	5.38	4899.04	0.12	0.05
P93165	Em protein OS Glycine max PE 4 SV 1	11 484	5.35	4895.39	0.08	0.03
Q04672	Sucrose-binding protein OS Glycine max GN SBP PE 1 SV 1	60 484	6.42	4608.47	3.36	1.46
C6SVM2	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17 367	9.48	4510.81	0.52	0.22
Q07CZ3	Glyceraldehyde 3 dehydrogenase C subunit OS Glycine max PE 2 SV 1	36 701	6.84	4077.10	0	0
C6SWV3	Putative uncharacterized protein OS Glycine max PE 2 SV 1	27 618	5.66	3894.10	0.71	0.31
C6TB70	Putative uncharacterized protein OS Glycine max PE 2 SV 1	24 404	6.50	3485.42	0.73	0.31
Q9XES8	Seed maturation protein PM28 OS Glycine max GN PM28 PE 4 SV 1	9506	4.46	3193.13	0.18	0.08
C6T0L2	Putative uncharacterized protein OS Glycine max PE 4 SV 1	11 134	6.37	3078.30	1.15	0.49
038IW8	Triosephosphate isomerase OS Glycine max PE 2 SV 1	27 187	5.81	2909.14	0.20	0.08
Q9SWB2	Seed maturation protein PM41 OS Glycine max GN PM41 PE 4 SV 1	8172	4.66	2896.55	0.21	0.091
Q42795	β-Amylase OS Glycine max PE 1 SV 1	56 036	5.18	2892.25	2.01	0.87
039871	Late embryogenesis abundant protein OS Glycine max GN MP2 PE 2 SV 1	50 613	6.29	2760.77	4.41	1.91
C6T0B5	Putative uncharacterized protein OS Glycine max PE 2 SV 1	13 998	5.69	2535.70	0.43	0.19
C6SVR5	Putative uncharacterized protein OS Glycine max PE 2 SV 1	23 888	5.63	2489.41	0.42	0.18
P00330	ALCOHOL DEHYDRUGENASE I EC 1 1 1 1	36 668	6.27	2351.86	0.91	0.39
004450	Putative uncharacterized protein US Glycine max PE 2 SV 1	2/ 031	6.45	2284.27	0.54	0.23
064458	GIV m Bd 30K allergen US Glycine max GN P34 PE 2 SV 1	42 / 30	5.56	2154./1	3.44	1.49
COTDOZ	Putative uncharacterized protein OS Glycine max PE 2 SV 1	31 008	7.50	2149.04	0.04	0.02
CETRET	Putative uncharacterized protein OS Chycine max PE 2 SV 1	27 701	5.05	1700.65	0.33	0.14
	Flongation factor 1 g-OS Glycing may GN FE 1A DE 2 SV 1	49 365	9.22	1653.60	1.33	0.14
C6T072	Putative uncharacterized protein OS Glycine may PE 2 SV 1	17 442	5.20	1651.00	0.31	0.30
C6S\//E0	Putative uncharacterized protein OS Glycine max FE 2 SV 1	17 355	5.20	1615.87	0.01	0.06
P26413	Heat shock 70 kDa protein OS Glycine may GN HSP70 PF 3 SV 1	70 835	5.20	1481 25	1 77	0.00
Q6RIB6	Malate dehydrogenase OS Glycine max PE 2 SV 1	35 504	6.34	1466.79	0.45	0.19

Entry	Description	MW (Da)	p/ (pH)	PLGS score	Amount (ng)	% of TSP
C6TK76	Putative uncharacterized protein OS Glycine max PE 2 SV 1	41 507	5 15	1249 91	0 13	0.06
C6TGM9	Putative uncharacterized protein OS Glycine max PE 2 SV 1	22 317	5.92	1194.81	0.11	0.05
A1KR24	Dehvdrin OS Glycine max GN LEA 2 D11 PE 3 SV 1	25 369	6.11	1148.55	0.80	0.35
C6T920	Phosphoglycerate kinase Fragment OS Glycine max PE 2 SV 1	25 296	9.71	1114.19	0.24	0.10
Q84V19	Sucrose-binding protein 2 OS Glycine max GN SBP2 PE 2 SV 1	55 740	6.10	1069.19	0.30	0.13
Q9SP11	Sucrose-binding protein homolog S 64 OS Glycine max GN SBP PE 2 SV 1	55 799	6.31	1005.86	0.23	0.10
Q96450	14-3-3-like protein A OS Glycine max GN GF14A PE 2 SV 1	29 030	4.49	910.00	0.40	0.17
C6T9C2	Putative uncharacterized protein OS Glycine max PE 2 SV 1	34 556	5.80	842.04	0.85	0.36
Q71EW8	Methionine synthase OS Glycine max PE 2 SV 1	84 229	5.88	833.61	1.34	0.58
C6K8D1	Seed biotinylated protein 68 kDa isoform OS Glycine max PE 2 SV 1	67 906	6.14	781.99	6.58	2.86
C6SZX7	Glutathione peroxidase OS Glycine max PE 2 SV 1	18 491	6.94	737.67	0.16	0.07
C6T1V2	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17 729	6.39	727.80	0.18	0.08
C6TNU2	Putative uncharacterized protein OS Glycine max PE 2 SV 1	47 497	5.66	684.35	0.05	0.02
P27066	Ribulose bisphosphate carboxylase large chain OS Glycine max GN rbcL PE 1 SV 3	52 576	5.97	675.63	0.54	0.23
C6TB98	Putative uncharacterized protein OS Glycine max PE 2 SV 1	33 906	5.50	664.43	0.38	0.16
C6T8D8	Fructose bisphosphate aldolase Fragment OS Glycine max PE 2 SV 1	28 937	7.11	633.63	0.31	0.13
C6T4R9	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17 656	10.15	584.85	0.32	0.14
C6SZN7	Putative uncharacterized protein OS Glycine max PE 4 SV 1	12 980	5.14	576.56	0.16	0.06
C6TLT3	Putative uncharacterized protein OS Glycine max PE 2 SV 1	29 708	10.23	557.49	0.09	0.04
C6TMG1	Fructose bisphosphate aldolase OS Glycine max PE 2 SV 1	38 315	7.34	534.53	0.30	0.13
C6T049	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17 988	5.38	529.73	0.11	0.05
Q6RIB7	Enolase OS Glycine max PE 2 SV 1	47 689	5.14	518.47	0.53	0.23
C6T4Z6	Putative uncharacterized protein OS Glycine max PE 2 SV 1	15 883	10.54	502.43	0.04	0.01
C6SVT0	Putative uncharacterized protein OS Glycine max PE 2 SV 1	18 011	6.93	460.71	0.09	0.03
Q39839	Nucleoside diphosphate kinase 1 OS Glycine max PE 2 SV 1	16 432	5.88	458.52	0	0
C6SYU0	Putative uncharacterized protein OS Glycine max PE 2 SV 1	18 273	8.62	422.07	0.15	0.06
C6SZN6	Putative uncharacterized protein OS Glycine max PE 2 SV 1	17 935	5.50	386.40	0.04	0.02
C6TG05	Putative uncharacterized protein OS Glycine max PE 2 SV 1	40 355	6.52	381.64	0.32	0.13
C6K8D0	Trypsin inhibitor 26 kDa isoform OS Glycine max PE 2 SV 1	25 930	8.40	377.51	0.27	0.11
C6T1R3	Putative uncharacterized protein OS Glycine max PE 2 SV 1	25 133	5.96	362.56	0.18	0.08
Q9SPB8	Malate dehydrogenase OS Glycine max GN Mdh1 PE 3 SV 1	36 119	8.22	330.78	0.20	0.08
QOGJJ9	ACP thioesterase protein Fragment OS Glycine max GN FATB1b PE 4 SV 1	22 492	5.48	318.69	0.81	0.35
Q9SWB4	Poly ADP ribose polymerase 3 OS Glycine max GN PARP3 PE 2 SV 1	91 630	5.26	316.88	0.83	0.36
C6TNI8	Putative uncharacterized protein Fragment OS Glycine max PE 2 SV 1	20 580	5.56	313.77	0.32	0.14
P29530	P24 oleosin isoform A OS Glycine max PE 2 SV 2	23 487	9.05	298.62	0.30	0.13
CESXUU	Putative uncharacterized protein US Glycine max PE 2 SV 1	2/ /21	6.81	295.30	0.23	0.10
C6SW/9	40S ribosomal protein S12 US Glycine max PE 2 SV 1	14 /88	5.17	249.56	0.09	0.04
P28551	Tubulin β -chain Fragment US Glycine max GN TUBB PE 2 SV 2	45 /21	5.55	246.58	0.09	0.04
020001	Putative uncharacterized protein US Glycine max PE 2 SV 1	51 462	5.25	242.40	0.57	0.25
U39801	51 KDa seed maturation protein US Glycine max PE 2 SV 1	50 951	6./4 0.15	239.27	0.24	0.10
COTCAC	Putative uncharacterized protein US Glycine max PE 2 SV 1	15 908	0.15	237.95	0.04	0.01
LOIGAD	Putative uncharacterized protein US Glycine max PE 2 SV 1	34 107	4.80	227.22	0.09	0.03
000////5	Putative uncharacterized protein US Glycine max PE 2 SV I	23 473	5.//	219.11	0.14	0.06
	Basic /S globulin 2 US Glycine max PE 1 SV 1	4/ 1/4	8.17 9.00	205.53	0 10	0.00
	Putative uncharacterized protein US Glycine max PE 2 SV 1	13 300 E0 EE4	0.90 4.0E	190.49	0.19	0.08
	Protein disulide isomerase US Giveine max GN PDIL I PE 3 SV 1	00 004	4.90	190.13	0.52	0.22
	Vunita truncia protococo inhibitor OS Chucino mov DE 2 SV 1	40 000	7.70	109.20	0.20	0.11
	Ruthiz trypsin protease initialition US Glycine max PE 2 SV 1	22 001	0.00 E 96	177.00	0 15	0.06
DE 4774	Coll division cycle protein 48 homolog OS Chusing may CN CDC48 PE 2 SV 1	37 948	0.00 5.00	160.02	0.10	0.00
Г 04//4 ССТОУБ	Putative uncharacterized protein OS Glycine max PE 2 SV 1	12 552	5.00	160.00	0.02	0.50
CESADI	Putative uncharacterized protein OS Glycine max PE 2 SV 1	43 333	0.77 11.20	166.69	0.22	0.09
	Putative uncharacterized protein OS Glycine max PE 2 SV 1	36 175	4.66	162.05	0.00	0.02
C6T262	Putative uncharacterized protein OS Clycine max PE 2 SV 1	22 272	7 20	162.20	0.10	0.07
CGTNIO2	Putative uncharacterized protein OS Glycine max PE 2 SV 1	22 373	10.62	1/1/ 01	0.66	0.13
C6TL/6	Putative uncharacterized protein OS Glycine max FE 2 SV 1	29 582	5 75	144.31	0.00	0.23
	Transcription factor h7IP129 Fragment OS Glucino may GN h7IP129 PE 2 SV 1	20 602	9.60	1/12.01	0.77	0.33
09SP.16	Maturation protein pPM32 OS Glycine max GN PM32 PF 2 SV 1	18 871	5.31	135.95	0.24	0.10
			0.01		v i	00

Tah	le 2	Continued
Iab	IC Z.	Commueu

Entry	Description	MW (Da)	р <i>I</i> (рН)	PLGS score	Amount (ng)	% of TSP
C6TG88	Putative uncharacterized protein Fragment OS Glycine max PE 2 SV 1	17 907	4.58	131.27	1.35	0.58
C6ZRP9	Pti1 kinase-like protein OS Glycine max PE 2 SV 1	34 932	8.93	130.00	0.10	0.04
Q7XAC5	Embryo-specific urease OS Glycine max PE 2 SV 1	90 099	5.61	128.19	0.45	0.19
C6TJD3	Putative uncharacterized protein OS Glycine max PE 2 SV 1	35 726	7.61	122.80	0.15	0.06
022518	40S ribosomal protein SA OS Glycine max PE 2 SV 1	33 885	4.90	122.27	0.23	0.10
C6TGJ9	Putative uncharacterized protein OS Glycine max PE 2 SV 1	28 237	11.13	117.53	0.04	0.01
Q0PJB9	MYB transcription factor MYB131 Fragment OS Glycine max GN MYB131 PE 2 SV 1	36 138	9.08	111.34	0.70	0.30
Q8L7J4	Pyruvate kinase OS Glycine max PE 2 SV 1	55 280	7.08	109.98	0.22	0.09
C6T520	Putative uncharacterized protein OS Glycine max PE 2 SV 1	18 088	5.80	109.35	0.20	0.08
C6T6B2	Putative uncharacterized protein OS Glycine max PE 2 SV 1	19 918	5.16	104.24	0.15	0.06
C6SY64	Proteasome subunit β -type OS Glycine max PE 2 SV 1	24 533	7.04	103.56	0	0
C6T470	Putative uncharacterized protein OS Glycine max PE 2 SV 1	27 220	4.52	101.06	0.07	0.03
B0M1A8	Peroxisomal aminotransferase Fragment OS Glycine max PE 2 SV 1	31 458	6.0	100.99	0.37	0.16

the elevated energy MS mode, the collision energy was ramped from 12 to 45 eV during each 1.5 s spectrum. The radiofrequency that was applied to the quadrupole mass analyzer was adjusted so that the ions from m/z 50 to 2000 were efficiently transmitted, which ensured that any ions less than m/z 50 that were observed in the LC-MS data only arose from dissociations in the TRAP T-wave collision cell.

2.5 Data processing and protein identification

The MS data that were obtained from the LC-MS^E were processed and searched using the ProteinLynx Global Server (PLGS) version 2.4 (Waters). Proteins were identified using the software's embedded ion accounting algorithm and a search of the Glycine max database with MassPREP standards (MPDS) UniProtKB/Swiss-Prot digestion sequences (Phosphorylase - P00489 - PHS2_RABIT, Bovine Hemoglobin - P02070 - HBB_BOVIN, ADH -P00330 - ADH1_YEAST, BSA - P02769 - ALBU_BOVIN) (UniProtKB/Swiss-Prot-P78358-Cancer_ and CTAG testis_1) that were appended to the database. Identifications and quantitative data packaging were generated using dedicated algorithms [38, 47] and a search against a species-specific Uniprot database. The ion detection, clustering, and log-scale parametric normalizations were performed in PLGS with an Expression^E license installed. The intensity measurements were typically adjusted on these components, i.e. deisotoped and charge state-reduced EMRTs that were replicated throughout the complete experiment for the analysis at the EMRT cluster level. The fixed modification of carbamidomethyl-C was specified, and the variable modifications included were acetyl N-terminus, deamidation N, deamidation Q and oxidation M. Components were typically clustered with a 10 ppm mass precision and a 0.25-min time tolerance against the databasegenerated theoretical peptide ion masses with a minimum of one matched peptide. The alignment of elevated energy ions with low-energy precursor peptide ions was conducted with an approximate precision of 0.05 min. One missed cleavage site was allowed. The precursor and fragment ion tolerances were determined automatically. The protein identification criteria also included the detection of at least three fragment ions per peptide and the determination of at least one peptide per protein, and the identification of the protein was allowed with a maximum 4% false-positive discovery rate in at least three technical repeatability injections. Using protein identification replication as a filter, the false-positive rate was minimized because false-positive protein identifications, i.e. chemical noise, have a random nature and do not tend to replicate across injections. For the analysis of protein identification and quantification level, the observed intensity measurements were normalized to the intensity measurement of the identified peptides of the digested internal standard.

3 Results and discussion

3.1 Detection of recombinant CTAG protein

The CTAG amino acid sequence is shown in Fig. 1. The sample preparation from the TSP step to the nanoUPLC procedure is critical for a successful identification. The use of high-purity water and reagents is recommended because of the sensitivity of the technique. With the low peptide dilution that is provided by nanoUPLC, each peptide is carried into the mass spectrometer almost individually, which allows for the production of precursor and fragment spectra from almost every peptide in the sample. At this separation power, the number of spread chromatography peaks is reduced to a minimum. When nanoACQUITY is associated with MS^E acquisitions [39], the ion current is continuous, both precursors and product ions are acquired in parallel, the chromatographic peaks are sharpened as more points per peak are obtained, and there is high

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Table 3. Expression analysis comparison of control (Soy Cn) and expressed (Soy CTAG L3) samples. The first values correspond to the
ratio values from the samples. The log(e) of the ratio and its standard deviation are shown in parentheses. The p-values
corresponding to values from 0 to 1, in which 0–0.05 was considered down-regulated and 0.95–1.00 was considered up-
regulated, are shown in brackets

CFGIB HUMAN Cancer testis antigen 1 2886.4 Soy CTAG L3 Unique 02474 20476 Matriation pretein 0.5 Glycine max PE 2 SV 1 666.4 0.661.4 1.07.0 02101 SOYBN P24 decisin isoform A.0 S Glycine max PE 2 SV 1 372.6 0.61.2.3.1 1.07.0 02105 SOYBN Patative uncharacterized protein 0.5 Glycine max PE 2 SV 1 2281.66 0.25.1 1.44.0.67.1 1.06.1 042705 SOYBN Allergen 60 ym Bd 24K Fragment 0.5 Glycine max PE 2 SV 1 2281.66 0.25.1 1.42.0.67.1 0.31.0.67.1 0.43.1 1.06.1 1.01.1 1.01.0 1.01.0 0.02.1 0.25.1 1.40.07.1 0.25.1 1.40.07.1 0.24.1 0.25.1 1.40.07.1 0.24.1 0.25.1 1.03.1 0.25.1 1.03.1 0.25.1 1.03.1 0.25.1 1.03.1 0.25.1 1.03.1 0.25.1 1.03.1 0.25.1 1.03.1 0.25.1 0.25.1 0.25.1 0.25.1 0.25.1 0.25.1 0.25.1 0.25.1 0.25.1 0.25.1 0.25.1 0.25.1 0.25.1	Accession	Description	Score	Soy Cn: Soy CTAG L3
02447, SOYBN Maturation protein 05 Glycine max FE 2 SV 1 668.9 008 (- 251 - 023) [0] 02100, SOYBN Putative uncharacterized protein 05 Glycine max FE 2 SV 1 372.8 020 (- 1.45 - 0.69) [0] 022100, SOYBN uncharacterized protein 05 Glycine max FE 2 SV 1 281.80 023 (- 1.44 - 0.61) [0] 042700, SOYBN Lipoxogenasa 05 Glycine max GN lox/PE 2 SV 1 281.80 023 (- 1.44 - 0.61) [0] 042700, SOYBN Ned maturation protein 05 Glycine max FE 2 SV 1 780.80 023 (- 1.44 - 0.61) [0] 053WAS, SOYBN Putative uncharacterized protein 05 Glycine max FE 2 SV 1 787.86 023 (- 0.69) [0] 024 (- 0.81 + 0.24) [0] 053WAS, SOYBN Putative uncharacterized protein 05 Glycine max FE 2 SV 1 787.86 024 (- 0.81 + 0.21) [0] 023WAS, SOYBN Putative uncharacterized protein 05 Glycine max FE 2 SV 1 113 13.3 045 (- 0.62 + 0.21) [0] 024WAS, SOYBN Putative uncharacterized protein 05 Glycine max FE 2 SV 1 137.5 044 (- 0.63 + 0.62) [0.61] 0260S SOYBN Putative uncharacterized protein 05 Glycine max FE 2 SV 1 113 13.3 036 (- 0.63 + 0.63) [0.61] 0261D_2 SOYBN Putative uncharacterized protein 05 Glycine max FE 2 SV 1 114 0.63 + 0.64 [0.61]	CTG1B_HUMAN	Cancer testis antigen 1	2886.4	Soy CTAG L3 Unique
DLED1_SOVBN P24 elossin adorm A DS Bycine max PE 2 SV 2 2286.0 008 (-246_125) [0] D22120_SOVBN rx-Subunit of () conglycinin Fragment OS Bycine max PE 2 SV 1 372.8 023 (-1.44_0.01) [0] D2249_SOVBN Algeneration S Bycine max PE 2 SV 1 2281.86 025 (-1.44_0.01) [0] D3240_SOVBN Algeneration SC Bycine max PE 2 SV 1 2281.86 025 (-1.34_0.03) [0] D3240_SOVBN Seed maturition protein PMA OS Glycine max PE 2 SV 1 2786.85 0.31 (-0.97_0.04) [0] DSXX3_SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2786.85 0.31 (-0.86_0.04) [0] DSXX3_SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 113.13 0.64 (-0.64_0.02) [0] CATHER_SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.64 0.64 (-0.64_0.02) [0] CATHER_SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.85 0.54 (-0.62_0.04) [0] CATHER_SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.86 0.64 (-0.64_0.03) [0] CATHER_SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.86 0.64 (-0.44_0.54) [0] CATHER_SOVEN Put	Q42447_SOYBN	Maturation protein OS Glycine max GN MAT1 PE 2 SV 1	666.94	0.08 (-2.51±0.73) [0]
CGT8BD. SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 372.80 0.23 (-1.44 ± 0.68) [0] O22120. SOVEN Lipoxygenase OS Glycine max GN lox PE 2 SV 1 281.80 0.25 (-1.44 ± 0.67) [0] OAXWES, SOVEN Sade maturation protein PM3 OS Glycine max PE 2 SV 1 285.80 0.23 (-1.44 ± 0.67) [0] OSXNVS, SOVEN Patative uncharacterized protein OS Glycine max PE 2 SV 1 285.85 0.33 (-0.97 ± 0.48) [0] CSTN3S, SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 276.48 0.41 (-0.88 ± 0.24) [0] CSTN3S, SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.51 0.54 (-0.86 ± 0.62) [0.61] CSTN3S, SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.51 0.54 (-0.62 ± 0.04) [0] CSTN2S, SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.51 0.54 (-0.62 ± 0.04) [0] CSTN2S, SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.51 0.54 (-0.62 ± 0.04) [0] CSTN2S, SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.51 0.54 (-0.49 ± 0.53) [0.01] CSTN2S, SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 377.63 0.66	OLEO1_SOYBN	P24 oleosin isoform A OS Glycine max PE 2 SV 2	298.62	0.09 (-2.46±1.25) [0]
022120. SOVBN -c-Subunit of § complycinin Fragment 0S Glycine max PE 2 SV 1 2381.86 025 (-1.44.c.0.7) (0.0) 0240700. SOVBN Alergen Gly, m Bd 28K Fragment 0S Glycine max PE 2 SV 1 258.08 025 (-1.34.c.0.30) (0.0) 02L0LG, SOVEN Seed matrixotion protein PMA 0S Glycine max PE 2 SV 1 278.45 0.01 (-0.380.24) 02L0, SOVEN Putative uncharacterized protein 0S Glycine max PE 2 SV 1 278.45 0.04 (-0.380.24) 0430W2, SOVEN Putative uncharacterized protein 0S Glycine max PE 2 SV 1 131.13 0.44 (-0.880.24) 0430W2, SOVEN Putative uncharacterized protein 0S Glycine max PE 2 SV 1 131.13 0.44 (-0.680.24) 0430W2, SOVEN Putative uncharacterized protein 0S Glycine max PE 2 SV 1 307.81 0.54 (-0.680.27) 05102, SOVEN Tryssin inhibitor 2 & blo isoform 0S Glycine max PE 2 SV 1 307.81 0.54 (-0.480.27) 05102, SOVEN Putative uncharacterized protein 0S Glycine max PE 2 SV 1 111.12 0.64 (-0.480.27) 0525, SOVEN Putative uncharacterized protein 0S Glycine max PE 2 SV 1 111.42 0.64 (-0.440.24) 05102, SOVEN Putative uncharacterized protein 0S Glycine max PE 2 SV 1 111.12 258.68 0.7 (-0.380.	C6T8B0_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	372.96	$0.23~(-1.45\pm0.69)~[0]$
042780. SOVBN Lipsoxygenese 0S Glycine max GN los/PE 2 SV 1 2818.0 025 (-1.4±.0.57) 10 [084NK8, SOVBN Sede maturation protein PM34 0S Glycine max PE 2 SV 1 2858.5 0.33 (-0.97.0.48) [0] 0SXV3, SOVBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2858.5 0.33 (-0.97.0.48) [0] 0SXV3, SOVBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 276.86 0.42 (-0.89±.0.21) [0] 0SXV3, SOVBN Glastatise provides OS Glycine max PE 2 SV 1 1151.52 0.44 (-0.81±.0.21) [0] 0SXV3, SOVBN Glastatise provides OS Glycine max PE 2 SV 1 1311.33 0.45 (-0.8±.0.21) [0] 0SX00, SOVBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 307.51 0.54 (-0.8±.0.21) [0] 0SGL, SOVBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 307.51 0.54 (-0.8±.0.21) [0] 0SGL, SOVBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 307.51 0.54 (-0.4±.0.57) [0.05] 0SGL, SOVBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 207.64 0.74 (-0.4±.0.57) [0.01] 0SGL, SOVBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 207.65 0.73 (-0.4±.0.56) [0.1] <t< td=""><td>022120_S0YBN</td><td>$\alpha\text{-Subunit}$ of β conglycinin Fragment OS Glycine max PE 2 SV 2</td><td>51 090.21</td><td>0.24 (-1.41±0.16) [0]</td></t<>	022120_S0YBN	$\alpha\text{-Subunit}$ of β conglycinin Fragment OS Glycine max PE 2 SV 2	51 090.21	0.24 (-1.41±0.16) [0]
0BAVRA_SOVEN Allergen Giv m Bd 28K Fragment 0S Glycine max PE 2 SV 1 500.00 0.25 (-1.38 ± 0.05 (0.05 0.05 ± 0.05 (0.05 0.05 ± 0.05 (0.05 0.05 ± 0.05 (0.05 ± 0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 ± 0.05 (0.05 ± 0.05 (0.05 ± 0.05 ± 0.05 (0.05 ± 0.05 ± 0.05 ± 0.05 ± 0.05 (0.05 ± 0.	Q42780_SOYBN	Lipoxygenase OS Glycine max GN lox7 PE 2 SV 1	2381.86	0.25 (-1.4±0.67) [0]
08LLG, SOYEN Seed maturation protein PM34 05 Glycine max PE 2 SV 1 786.5 0.33 (-0.97, -0.68) [0] 06XXV3 SOYEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 286.8 0.03 (-0.97, -0.68) [0] 06XXV3 SOYEN Futative uncharacterized protein OS Glycine max PE 2 SV 1 737.68 0.44 (-0.87, +0.01) [0.02 06XXV3 SOYEN Futative uncharacterized protein OS Glycine max PE 2 SV 1 1151.52 0.44 (-0.87, +0.01) [0.02 06XXV3 SOYEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 1377.51 0.54 (-0.82, +0.04) [0] 06XXV3 SOYEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 107.67.31 0.55 (-0.53, +0.04) [0] 06X102 SOYEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 114.2 0.64 (-0.44, -0.64) [0.10 06X120 SOYEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 114.2 0.64 (-0.44, -0.64) [0.10 06X120 SOYEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 114.2 0.64 (-0.44, -0.64) [0.10 06X120 SOYEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 114.2 0.64 (-0.44, -0.64) [0.10] 06X120 SOYEN Malate dehydrogenase oS Glycine max PE 2 SV 1 120.05 120.05 </td <td>Q9AVK8_SOYBN</td> <td>Allergen Gly m Bd 28K Fragment OS Glycine max PE 2 SV 1</td> <td>5503.09</td> <td>0.25 (-1.38±0.36) [0]</td>	Q9AVK8_SOYBN	Allergen Gly m Bd 28K Fragment OS Glycine max PE 2 SV 1	5503.09	0.25 (-1.38±0.36) [0]
C6SXV3. S0YEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2868 0.39 (-0.85 (-0.84) C6TNJ3. S0YEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2764.85 0.44 (-0.88 (-0.24) C6TNJ3. S0YEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 1151.52 0.44 (-0.88 (-0.21) C6TNJ3. S0YEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 1131.33 0.45 (-0.8 (-0.21) C6TUJ2. S0YEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 307.51 0.54 (-0.62 (-0.54) C6TUJ2. S0YEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 218.05 (-0.59	Q9LLQ6_SOYBN	Seed maturation protein PM34 OS Glycine max GN PM34 PE 2 SV 1	7863.56	0.38 (-0.97±0.48) [0]
C6TX88 S0YEM Putative uncharacterized protein 0S Glycine max PE 2 SV 1 2748.5 0.41 (-0.88 ± 0.24) (0.0 C6ZX7. SOYEM F-Amylase OS Glycine max PE 3 SV 1 11315.2 0.44 (-0.81 ± 0.21) (0) C6XDD. SOYEM P-trative uncharacterized protein 0S Glycine max PE 2 SV 1 1311.3 0.45 (-0.82 ± 0.21) (0) C6XDD. SOYEM Trynsin inhibitor 28 kDa isoform OS Glycine max PE 4 SV 1 307.31 0.54 (-0.62 ± 0.41) (0) C6TUB. SOYEM Putative uncharacterized protein 0S Glycine max PE 2 SV 1 307.31 0.54 (-0.42 ± 0.41) (0) C6TAB. SOYEM Putative uncharacterized protein 0S Glycine max PE 2 SV 1 1114.2 0.54 (-0.42 ± 0.54) (0) C6TAB. SOYEM Putative uncharacterized protein 0S Glycine max PE 2 SV 1 1114.2 0.54 (-0.42 ± 0.54) (0) C6TAB. SOYEM Matate dehydrogenase 0S Glycine max PE 2 SV 1 2211 07 (-0.36 ± 0.13) (0) 225 SOYEM ZS SOYEM Attact enthyrogenesis abundant protein 0S Glycine max GN MP2 PE 2 SV 1 2700.78 0.74 (-0.32 ± 0.24) (0.10) ZSS SOYEM Patate enthyrogenesis abundant protein 0S Glycine max FE 2 SV 1 180.65 0.75 (-0.22 ± 0.51) (0.16) ZSS SOYEM Patate enthyrogenesis abundant protein 0S Glycine max FE 2 SV 1 180.66 0.75 (-0.22 ± 0.5) (0.16)	C6SXV3_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	269.85	0.39 (-0.95±0.68) [0]
C6S2X7.S0YBN Giutathione peroxidase 0.5 Glycine max PE 2 SV 1 77.88 0.42 (-0.88 ± 0.61) [0.02) 04UW3_S0YBN P-Amyase 0.5 Glycine max PE 2 SV 1 1131.33 0.44 (-0.81 ± 0.21) [0] 0CKSDD_SOYBN Putative uncharacterized protein 0.5 Glycine max PE 2 SV 1 307.81 0.55 (-0.58 ± 0.26) [0.01] 0CKSDD_SOYBN Putative uncharacterized protein 0.5 Glycine max PE 4 SV 1 307.81 0.55 (-0.58 ± 0.26) [0.01] 1BEC2_SOYBN Putative uncharacterized protein 0.5 Glycine max PE 2 SV 1 28.09 0.64 (-0.44 ± 0.54) [0.1] 1CTABSOYBN Putative uncharacterized protein 0.5 Glycine max PE 2 SV 1 722.11 0.7 (-0.36 ± 0.19) [0] CGTABSOYBN Malate dehydrogeness 0.5 Glycine max PE 2 SV 1 720.11 0.7 (-0.36 ± 0.19) [0] 2SS_SOYEN ALCOHOL DEHYDROGENASE I EC 1 1 1 1 2351.86 0.7 (-0.36 ± 0.19) [0] 2SS_SOYEN Z4 alousin IOS Glycine max PE 2 SV 1 720.02 74 (-0.32 ± 0.19) [0] 2SS_SOYEN P24 aloesin isoform B OS Glycine max PE 2 SV 1 754.03 0.75 (-0.22 ± 0.46) [0.11] 2SS_SOYEN P24 aloesin isoform B OS Glycine max PE 2 SV 1 754.03 0.75 (-0.22 ± 0.62) [0.16] 0THOL SS_SOYEN Malate dehydrogenas	C6TN36_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	2764.85	0.41 (-0.88±0.24) [0]
Q4U3W3_SOVEN Ø-Amylase OS Glycine max PE 3 SV 1 1151.52 0.44 (-0.81 ± 0.21) [0] CGTDES_SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 131.13.3 0.45 (-0.82 ± 0.21) [0] CGTDES_SOVEN Putative uncharacterized protein OS Glycine max PE 4 SV 1 307.8.1 0.55 (-0.59 ± 0.36) [0.01] DEGTAS_SOVEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 1167.2. 0.57 (-0.57 ± 0.057 ±	C6SZX7_SOYBN	Glutathione peroxidase OS Glycine max PE 2 SV 1	737.68	0.42 (-0.86±0.61) [0.02]
C6TDFS_S0YBN Putative uncharacterized protein 05 Glycine max PE 2 SV 1 1131.33 0.45 0.64 0.62 0.27 0.54 0.63 0.64 0.62 0.64 0.63 0.64 0.63 0.64 0.63 0.64 0.63 0.65 0	Q4U3W3_SOYBN	β-Amylase OS Glycine max PE 3 SV 1	1151.52	0.44 (-0.81±0.21) [0]
CKK8DD, SOYBN Trypsin inhibitor 28 kDa isaform DS Glycine max PE 2 SV 1 377.51 0.54 (-0.62±0.4) [0] CGTUL2_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 307.61 0.54 (-0.62±0.4) [0] CGTU2_SOYBN Bowman Birk-type proteinase inhibitor C II OS Glycine max PE 2 SV 1 256.00 0.64 (-0.44±0.51) [0] CGTS2D_SOYBN Phosphodycerate kinase fragment OS Glycine max PE 2 SV 1 1114.2 0.64 (-0.44±0.51) [0] CGTS2D_SOYBN Allate dehydrogenase OS Glycine max PE 2 SV 1 72.11 0.7 (-0.36±1.03) [0] 2SS_SOYEN Zalate mbryogenesis abundant protein OS Glycine max RE 2 SV 1 256.866.75 0.73 (-0.3±0.13) [0] DLEOZ_SOYEN Proteose bisphosphate aldolase OS Glycine max PE 2 SV 1 150.50 0.75 (-0.29±0.46) [0.11] CGTLMSD_SOVEN Fructose bisphosphate aldolase OS Glycine max PE 2 SV 1 534.53 0.76 (-0.29±0.46) [0.12] DCTLWM_SOVEN Fructose bisphosphate actorbxylas large chain OS Glycine max PE 2 SV 1 1368.07 (-0.24±0.51) [0.16] DCTLWM_SOVEN Matate dehydrogenase OS Glycine max PE 2 SV 1 1368.07 (-0.24±0.51) [0.16] DCTLWM_SOVEN Mutate dehydrogenase OS Glycine max PE 2 SV 1 1368.07 (-0.24±0.51) [0.16] DCTLWM_SOVEN Mu	C6TDF5_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	11 311.33	0.45 (-0.8±0.27) [0]
CETOL2_SOYEN Putative uncharacterized protein 0S Glycine max PE 4 SV 1 3078.31 0.55 (0.59±0.39) [0.01] IBBCZ_SOYEN Bowman Birk-type proteinase inhibitor C II 0S Glycine max PE 2 SV 1 256.09 0.64 (0.44±0.54) [0.1] CETAB0_SOYEN Putative uncharacterized protein 0S Glycine max PE 2 SV 1 721.01 0.64 (0.44±0.51) [0.2] CETNAB_SOVEN Malate dehydrogeness 0S Glycine max PE 2 SV 1 722.11 0.7 (0.36±1.38) [0.19] ADH1_YEAST ALCOHOL DEHYDROGENASE I EC 1 1 1 255.18 0.7 (0.36±0.19) [0] 258 SOYEN Zabumin OS Glycine max PE 1 SV 2 26866.75 0.73 (0.3±0.11) [0] 258 JOYEN Late embryogenesis abundant protein OS Glycine max PE 2 SV 1 256.05 0.73 (0.2±0.24) [0.10] 0.1E02_SOYEN P24 eleosin isoform B 0.5 Glycine max PE 2 SV 1 156.35 0.75 (0.2±0.24) [0.10] 0.1E02_SOYEN Malate dehydrogeness 0.5 Glycine max PE 2 SV 1 136.86 0.77 (0.2±0.24) [0.10] 0.25605_SOYEN Malate dehydrogeness 0.5 Glycine max PE 2 SV 1 368.61 0.77 (0.2±0.24) [0.10] 0.23601_SOYEN Paterize and protein 0S Glycine max PE 2 SV 1 368.61 0.77 (0.2±0.24) [0.10] 0	C6K8D0_SOYBN	Trypsin inhibitor 26 kDa isoform OS Glycine max PE 2 SV 1	377.51	0.54 (-0.62±0.4) [0]
IBBCZ_SOYEN Bowman Birk-type proteinase inhibitor C III 05 Glycine max PE 2 SV 1 257 (-0.57±0.09) [0] CGTAGA_SOYEN Putative uncharacterized protein 05 Glycine max PE 2 SV 1 1114.2 0.64 (-0.44±0.54) [0].1 CGTAGA_SOYEN Malate dehydrogenase 05 Glycine max PE 2 SV 1 722.11 0.7 (-0.36±0.13) [0.02] CGTAGA_SOYEN Malate dehydrogenase 05 Glycine max PE 2 SV 1 726.06 7.7 (-0.36±0.13) [0] ZSS_SOYEN ZA LOCHOL DEHYDROGENASE I EC 111 1 2251.6 0.7 (-0.36±0.13) [0] ZSS_SOYEN L24 aleusini soform B OS Glycine max PE 2 SV 1 2760.78 0.74 (-0.3±0.1) [0] CGTABA_SOYEN P24 oleosini soform B OS Glycine max PE 2 SV 1 534.53 0.75 (-0.22±0.46) [0.11] CGTIEMS_SOYEN P24 oleosin isoform B OS Glycine max PE 2 SV 1 833.61 0.76 (-0.22±0.26) [0.24] ORIEMS_SOYEN Malate dehydrogenase 3 OS Glycine max PE 2 SV 1 1466.79 0.77 (-0.22±0.27) [0.04] CATEWASOYEN Poly ADP ribose polymerase 3 OS Glycine max PE 2 SV 1 238.20 0.78 (-0.22±0.33) [0.01] CGTEMS_SOYEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 238.20 0.78 (-0.22±0.33) [0.02] CGTEMS_SOYEN Naturation protein PM22 OS Glycine	C6T0L2_SOYBN	Putative uncharacterized protein OS Glycine max PE 4 SV 1	3078.31	0.55 (-0.59±0.36) [0.01]
CGTABO_SOVBN Putative uncharacterized protein QS Glycine max PE 2 SV 1 258.09 0.64 (-0.44 ± 0.54) [0.1] CGTAD_SOVBN Phosphoglycerate kinase Fragment OS Glycine max PE 2 SV 1 1114.2 0.64 (-0.44 ± 0.54) [0.19] ADH1_YEAST ALCOHOL DEHYDROGENASE I EC 1 1 1 2351.86 0.7 (-0.36 ± 1.38) [0.19] ADM1_YEAST ALCOHOL DEHYDROGENASE I EC 1 1 1 2351.86 0.7 (-0.36 ± 1.38) [0.19] CGTABO_SOVBN Late embryogenesis abundant protein OS Glycine max GN MP2 PE 2 SV 1 2760.78 0.74 (-0.32 ± 0.13) [0] CGTMAE_SOVBN P24 oleosin isoform B OS Glycine max PE 2 SV 1 150.05 0.75 (-0.29 ± 0.16) [0.16] OCTMOT_SOVEN Mathionine synthase OS Glycine max PE 2 SV 1 833.61 0.76 (-0.27 ± 0.26) [0.16] OTIEME_SOVEN Mathionine synthase OS Glycine max PE 2 SV 1 1466.79 0.77 (-0.24 ± 0.36) [0.11] OSTIGOS_SOVEN Poly ADP ribose polymerase 3 OS Glycine max PE 2 SV 1 239.80 0.79 (-0.24 ± 0.36) [0.1] OSTIGOS_SOVEN Poly ADP ribose polymerase 3 OS Glycine max PE 2 SV 1 316.88 0.79 (-0.24 ± 0.36) [0.1] OSTIGOS_SOVEN Poly ADP ribose polymerase 3 OS Glycine max PE 2 SV 1 239.28 0.79 (-0.24 ± 0.36) [0.1] OSTIGOS	IBBC2_SOYBN	Bowman Birk-type proteinase inhibitor C II OS Glycine max PE 1 SV 2	19 673.12	0.57 (-0.57±0.09) [0]
CGT320_SOVBN Phosphoglycerate kinase Fragment 05 Glycine max PE 2 SV 1 1114.2 0.64 (-0.44 + 0.37) [0.02] CGTNA8_SOVBN Malate dehydrogenase 05 Glycine max PE 2 SV 1 722.11 0.7 (-0.36 ± 1.38) [0.19] ALCDHOL DEHYDROGENASE 1 EC 11 1 1 2351.86 0.7 (-0.38 ± 0.19) [0] 2255 SOVBN 225 albumin 05 Glycine max PE 1 SV 2 2866.75 0.73 (-0.31 ± 0.1) [0] OLEO2_SOVBN P24 oleosin isoform B OS Glycine max PE 2 SV 1 256.65 0.75 (-0.29 ± 0.46) [0.11] CGTMAB_SOVBN P24 oleosin isoform B OS Glycine max PE 2 SV 1 333.61 0.76 (-0.27 ± 0.26) [0.03] OTEWS_SOVBN Malate dehydrogenase 0S Glycine max PE 2 SV 1 336.80 0.77 (-0.26 ± 0.27) [0.16] OTEWS_SOVBN Malate dehydrogenase 0S Glycine max PE 2 SV 1 316.88 0.79 (-0.24 ± 0.36) [0.11] OSB01_SOVBN Flutaviacterized protein 0S Glycine max PE 2 SV 1 318.64 0.8 (-0.22 ± 0.36) [0.29] OSBVDS_SOVBN Poly ADP ribose polymerase 3 OS Glycine max PE 2 SV 1 318.64 0.8 (-0.22 ± 0.36) [0.29] OSBVDS_SOVBN Rutaviation protein DYG/SO CS Glycine max PE 2 SV 1 318.64 0.8 (-0.22 ± 0.36) [0.29] OSBVDS_SOVBN Rutaviation protein PM25 OS Glycine max PE 2 SV 1	C6TA60_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	258.09	$0.64~(-0.44\pm0.54)~[0.1]$
CBTMAB. SOYBN Malate dehydrogenase 0.S Glycine max PE 2 SV 1 722.11 0.7 (-0.38 ± 1.38) [0.19] DAHL YEAST ALCOHOL DEHYDROGENASE I EC 11 1 1 2351.86 0.7 (-0.38 ± 1.38) [0.19] QSS SOYBN 2S albumin OS Glycine max PE 1 SV 2 26.666.75 0.73 (-0.31 ± 0.11) QSS SOYBN Late embryogenesis abundant protein OS Glycine max PE 2 SV 1 76.0.78 0.74 (-0.3± 0.13) DLEO2_SOYBN P24 olesein isoform B OS Glycine max PE 2 SV 1 534.53 0.75 (-0.29± 0.5) 0.16) DTIEWA SOYBN Matetionine synthase OS Glycine max PE 2 SV 1 534.53 0.76 (-0.29± 0.21) 0.16) GRIBE_SOYBN Matate dehydrogenase OS Glycine max PE 2 SV 1 336.16 0.76 (-0.22± 0.21) 0.10 QSRIBE_SOYBN Poly ADP ribose polymerase 3 OS Glycine max GN PARP3 PE 2 SV 1 381.64 0.6 (-0.22± 0.31) 10.19 QSRIDE_SOYBN Poly ADP ribose polymerase 3 OS Glycine max GN AARP3 PE 2 SV 1 381.64 0.6 (-0.22± 0.33) 10.19 QSRIDE_SOYBN Poly ADP ribose polymerase 3 OS Glycine max GN AARP 2 PE 3 SV 1 381.64 0.8 (-0.22± 0.33) 10.19 QSRIDE_SOYBN Puly ADP ribose polymerases 0 S Glycine max PE 2 SV 1 381.64	C6T920_SOYBN	Phosphoglycerate kinase Fragment OS Glycine max PE 2 SV 1	1114.2	0.64 (-0.44±0.37) [0.02]
ADH1_YEAST ALCOHOL DEHVDROGENASE I EC 1 1 1 2251 207 (-0.3 ± 0.19) [0] 2SS_SOYBN 2S albumin OS Glycine max PE 1 SV 2 2666.75 0.73 (-0.3 ± 0.1) [0] 028671_SOYBN Late embryogenesis abundant protein OS Glycine max RE 2 SV 1 2760.78 0.74 (-0.3 ± 0.1) [0] 01E02_SOYBN P24 olessin stoform B OS Glycine max PE 2 SV 1 534.53 0.75 (-0.29 ± 0.6) [0.16] 071EW8_SOYBN Matte dehydrogenase OS Glycine max PE 2 SV 1 534.53 0.75 (-0.29 ± 0.6) [0.16] 071EW8_SOYBN Malate dehydrogenase OS Glycine max RD PARP3 PE 2 SV 1 316.88 0.76 (-0.22 ± 0.42) [0.13] 03801_SOYBN 51 kDa seed maturation protein OS Glycine max GN PARP3 PE 2 SV 1 316.84 0.8 (-0.22 ± 0.23) [0.04] 03801_SOYBN Flutative uncharacterized protein OS Glycine max GN PARP3 PE 2 SV 1 381.64 0.8 (-0.22 ± 0.43) [0.03] 03801_SOYBN Riutous entracterized protein OS Glycine max GN PM32 PE 2 SV 1 381.64 0.8 (-0.22 ± 0.45) [0.19] 03801_SOYBN Maturation protein PM32 OS Glycine max GN PM32 PE 2 SV 1 381.64 0.8 (-0.22 ± 0.45) [0.29] 03874_SOYBN Alcohol dehydrogenase Fragment OS Glycine max FE 1 SV 2 1878.71 0.83 (-0.19 ± 0.10) [0.20]	C6TNA8_SOYBN	Malate dehydrogenase OS Glycine max PE 2 SV 1	722.11	0.7 (-0.36±1.38) [0.19]
2SS_SOVBN 2S albumin 0.5 Glycine max PE 1 SV 2 26 866.75 0.73 (-0.31 ± 0.1) [0] 0.39871_SOVBN Late embryogenesis abundant protein OS Glycine max RP 2 SV 1 2760.78 0.74 (-0.31 ± 0.1) [0] 0.ED2_SOVBN P24 olesin isoform B 0.S Glycine max PE 2 SV 1 150.05 0.75 (-0.29 ± 0.46) [0.11] 0.CED2_SOVBN P24 olesin isoform B 0.S Glycine max PE 2 SV 1 534.53 0.75 (-0.29 ± 0.5) [0.16] 0.CED2_SOVBN Matte dehydrogenase 0.S Glycine max PE 2 SV 1 1466.79 0.77 (-0.26 ± 0.27) [0.04] 0.SRIBE_SOVBN Matte dehydrogenase 0.S Glycine max RE 2 SV 1 386.8 0.77 (-0.26 ± 0.27) [0.19] 0.39801_SOVBN Poly ADP ribose polymerase 3 0.S Glycine max RE 2 SV 1 386.4 0.02 ± 0.23) [0.19] 0.39801_SOVBN Poly ADP ribose polymerase 3 0.S Glycine max PE 2 SV 1 381.64 0.64 -0.22 ± 0.38) [0.13] 0.3801_SOVBN Poly ADP ribose polymerase 10.S Glycine max GN PM32 PE 2 SV 1 381.64 0.8 (-0.22 ± 0.38) [0.29] 0.3974_GOVBN Ribulose bisphosphate carboxylase large chain OS Glycine max PE 3 SV 1 1260.14 0.8 (-0.22 ± 0.48) [0.29] 0.32738_SOVBN Maturation protein pPM32 OS Glycine max GN PM32 PE 2 SV 1 397.0.3 (-0.19 ± 0.42) [0.24] <td< td=""><td>ADH1_YEAST</td><td>ALCOHOL DEHYDROGENASE I EC 1 1 1 1</td><td>2351.86</td><td>0.7 (-0.36±0.19) [0]</td></td<>	ADH1_YEAST	ALCOHOL DEHYDROGENASE I EC 1 1 1 1	2351.86	0.7 (-0.36±0.19) [0]
C39871_SOYEN Late embryogenesis abundant protein OS Glycine max PE 2 SV 1 2760.78 0.74 (-0.3 ± 0.13) [0] OLEO2_SOYEN P24 oleosin isoform B OS Glycine max PE 2 SV 1 534.53 0.75 (-0.29 ± 0.45) [0.11] CGTMG1_SOYEN Fructose bisphosphate aldolase OS Glycine max PE 2 SV 1 534.53 0.75 (-0.29 ± 0.55) [0.03] QGRIB6_SOYEN Malate dehydrogenase OS Glycine max PE 2 SV 1 316.88 0.77 (-0.26 ± 0.27) [0.04] PAPP2_SOYEN Poly ADP ribose polymerase 3 OS Glycine max FE 2 SV 1 316.84 0.77 (-0.24 ± 0.55) [0.13] GGRIG5_SOYEN Poly ADP ribose polymerase 3 OS Glycine max FE 2 SV 1 381.64 0.8 (-0.22 ± 0.38) [0.13] GGRIG5_SOYEN Putative uncharacterized protein OS Glycine max GN AM2 PE 3 SV 1 1266.14 0.8 (-0.22 ± 0.38) [0.29] QG2T38_SOYEN Alcohol dehydrogenase Fragemet OS Glycine max GN AM2 PE 3 SV 1 1260.14 0.8 (-0.22 ± 0.49) [0.19] IBBD2_SOYEN Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2 187.89.71 0.81 (-0.21 ± 0.42) [0.16] QGSIWA Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 1 1260.14 0.8 (-0.22 ± 0.49) [0.19] UBSD2_SOYEN Putative uncharacterized protein OS Glycine max PE 2 SV 1 776.55<	2SS_SOYBN	2S albumin OS Glycine max PE 1 SV 2	26 866.75	0.73 (-0.31±0.1) [0]
DLE02. SOVBN P24 oleosin isoform B 0S Glycine max PE 2 SV 1 150.05 0.75 (-0.29 ± 0.49) (0.11) C6TMG1_SOYBN Fructose bisphosphate aldolase 0S Glycine max PE 2 SV 1 833.61 0.76 (-0.27 ± 0.28) (0.03) OTIEWS_SOYBN Malate dehydrogenase 0S Glycine max PE 2 SV 1 833.61 0.76 (-0.27 ± 0.28) (0.03) ORRIBG_SOYBN Malate dehydrogenase 0S Glycine max PE 2 SV 1 1466.79 0.77 (-0.26 ± 0.27) (0.04) PARP3_SOYBN Foly ADP ribose polymerase 3 OS Glycine max GN PARP3 PE 2 SV 1 239.28 0.79 (-0.24 ± 0.53) (0.19) C39801_SOYBN Poly ADP ribose polymerase 3 OS Glycine max FE 2 SV 1 239.28 0.79 (-0.24 ± 0.53) (0.19) C6TG05_SOYBN Putative uncharacterized protein OS Glycine max FE 2 SV 1 381.64 0.8 (-0.22 ± 0.43) (0.03) OSSPL6_SOYBN Maturation protein PM23 OS Glycine max GN Adh 2 PE 3 SV 1 1250.64 0.8 (-0.22 ± 0.46) (0.19) USSPL6_SOYBN Bowman Birk-type proteinase inhibitor D1 ID S Glycine max FE 1 SV 2 1878.97 (0.81 (-0.21 ± 0.42) (0.16) USSFL8_SOYBN Alcohol dehydrogenase Fragment OS Glycine max PE 1 SV 2 1878.97 (0.81 (-0.21 ± 0.42) (0.16) USSFL8_SOYBN Triosephosphate isomerase OS Glycine max PE 1 SV 1 570.50 0.83 (-0.19 ± 0.14) (0.42) <td>Q39871_SOYBN</td> <td>Late embryogenesis abundant protein OS Glycine max GN MP2 PE 2 SV 1</td> <td>2760.78</td> <td>0.74 (-0.3±0.13) [0]</td>	Q39871_SOYBN	Late embryogenesis abundant protein OS Glycine max GN MP2 PE 2 SV 1	2760.78	0.74 (-0.3±0.13) [0]
C6TMG1_S0YBN Fructose bisphosphate aldolase OS Glycine max PE 2 SV 1 534.53 0.75 (-0.29 ± 0.5) [0.16] 071EW8_S0YBN Methionine synthase OS Glycine max PE 2 SV 1 833.61 0.76 (-0.27 ± 0.26) [0.07] 06RH65_S0YBN Poly ADP ribose polymerase 3 OS Glycine max PE 2 SV 1 1466.79 0.77 (-0.28 ± 0.27) [0.04] 078105_S0YBN Poly ADP ribose polymerase 3 OS Glycine max PE 2 SV 1 316.88 0.79 (-0.24 ± 0.36) [0.1] 039801_S0YBN Poly ADP ribose polymerase 3 OS Glycine max PE 2 SV 1 381.64 0.8 (-0.22 ± 0.33) [0.09] 039801_S0YBN Ribulose bisphosphate carboxylase large chain OS Glycine max GN rbcL PE 1 SV 3 675.63 0.8 (-0.22 ± 0.43) [0.19] 0385PJ6_S0YBN Maturation protein pPM32 OS Glycine max GN PM32 PE 2 SV 1 135.96 0.8 (-0.22 ± 0.46) [0.19] 045D2T38_S0YBN Maturation protein PM22 OS Glycine max GN PM26 PE 3 SV 1 1260.14 0.8 (-0.22 ± 0.46) [0.19] 045D2S0VBN Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2 1878.71 0.81 (-0.21 ± 0.42) [0.16] 0381W8_S0YBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 766.57 0.84 (-0.19 ± 0.14) [0.02] 0458W9_S0YBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 <	OLEO2_SOYBN	P24 oleosin isoform B OS Glycine max PE 2 SV 1	150.05	0.75 (-0.29±0.46) [0.11]
071EW8_SOYBN Methionine synthase 0S Glycine max PE 2 SV 1 833.61 0.76 (-0.27 ± 0.26) [0.03] 06RIB6_SOYBN Malate dehydrogenase 0S Glycine max PE 2 SV 1 316.89 0.77 (-0.26 ± 0.27) [0.04] PAPR3_SOYBN Poly ADP ribose polymerase 3 OS Glycine max PE 2 SV 1 316.88 0.79 (-0.24 ± 0.51) [0.19] 039801_SOYBN 51 kDa seed maturation protein OS Glycine max PE 2 SV 1 239.28 0.73 (-0.24 ± 0.51) [0.19] CGEDS_SOYBN Putative uncharacterized protein OS Glycine max CP 2 SV 1 381.64 0.8 (-0.22 ± 0.33) [0.09] 0382PJ6_SOYBN Maturation protein pPM32 OS Glycine max GN PM32 PE 2 SV 1 135.96 0.8 (-0.22 ± 0.46) [0.19] 0382PJ6_SOYBN Alcohol dehydrogenase Fragment OS Glycine max GN Adh 2 PE 3 SV 1 1260.14 0.8 (-0.22 ± 0.46) [0.19] 0382FAS_SOYBN Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2 18 789.71 0.83 (-0.19 ± 0.45) [0.24] 038W8_SOYBN Fuitow uncharacterized protein OS Glycine max PE 2 SV 1 209.14 0.83 (-0.19 ± 0.45) [0.24] 038W8_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2760.69 0.83 (-0.19 ± 0.41) [0.02] 058EVA_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 804.4 (-0.18	C6TMG1_SOYBN	Fructose bisphosphate aldolase OS Glycine max PE 2 SV 1	534.53	0.75 (-0.29±0.5) [0.16]
OGRIB6_SOYBN Malate dehydrogenase OS Glycine max PE 2 SV 1 1466.79 0.77 (-0.26 ± 0.27) [0.04] PARP3_SOYBN Poly ADP ribose polymerase 3 OS Glycine max GN PARP3 PE 2 SV 1 316.88 0.77 (-0.26 ± 0.27) [0.04] O38001_SOYBN Folk Da seed maturation protein OS Glycine max FE 2 SV 1 239.28 0.77 (-0.26 ± 0.27) [0.04] OGRIGO_SOYBN Putative uncharacterized protein OS Glycine max FE 2 SV 1 239.28 0.77 (-0.26 ± 0.27) [0.04] OGRIGO_SOYBN Putative uncharacterized protein OS Glycine max FE 2 SV 1 239.28 0.77 (-0.26 ± 0.27) [0.09] OGRIGO_SOYBN Maturation protein PM32 OS Glycine max GN PM32 PE 2 SV 1 381.64 0.8 (-0.22 ± 0.33) [0.09] OgST3B_SOYBN Alcohol dehydrogenase Fragment OS Glycine max GN PM26 PE 3 SV 1 1260.14 0.8 (-0.22 ± 0.42) [0.19] IBBDZ_SOYBN Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2 18 789.71 0.81 (-0.21 ± 0.42) [0.46] QBIWB_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2909.14 0.83 (-0.19 ± 0.18) [0.02] QBIWB_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 576.57 0.84 (-0.18 ± 0.74) [0.28] QBSEL0_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1	Q71EW8_SOYBN	Methionine synthase OS Glycine max PE 2 SV 1	833.61	0.76 (-0.27 ± 0.26) [0.03]
PARP3_SOYBN Poly ADP ribose polymerase 3 OS Glycine max GN PARP3 PE 2 SV 1 316.88 0.79 (-0.24 ± 0.36) [0.1] 0.39801_SOYBN 51 kDa seed maturation protein OS Glycine max PE 2 SV 1 239.28 0.79 (-0.24 ± 0.51) [0.19] C6TG05_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 381.64 0.8 (-0.22 ± 0.38) [0.3] D8EL_SOYBN Ribulose bisphosphate carboxylase large chain OS Glycine max GN rbcL PE 1 SV 3 675.63 0.8 (-0.22 ± 0.53) [0.29] 092738_SOYBN Maturation protein pPM32 OS Glycine max GN PM32 PE 2 SV 1 1260.14 0.8 (-0.22 ± 0.46) [0.19] 092738_SOYBN Alcohol dehydrogenase Fragment OS Glycine max GN Adh 2 PE 3 SV 1 1260.14 0.8 (-0.22 ± 0.46) [0.19] 092738_SOYBN Seed maturation protein PM26 OS Glycine max FE 1 SV 2 18789.71 0.81 (-0.21 ± 0.42) [0.16] 093EVA_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 970.59 0.83 (-0.19 ± 0.47) [0.28] 025EU_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 7690.69 0.83 (-0.19 ± 0.47) [0.28] 025EU_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 8024.35 0.84 (-0.18 ± 0.08) [0] 025EU_SOYBN Detydrin OS Glycine max FG 3 SV 1 164	Q6RIB6_SOYBN	Malate dehydrogenase OS Glycine max PE 2 SV 1	1466.79	0.77 (-0.26 ± 0.27) [0.04]
039801_SOYBN 51 kDa seed maturation protein OS Glycine max PE 2 SV 1 239.28 0.79 (-0.24±0.51) [0.19] C6TG05_SOYBN Putative uncharacterized protein OS Glycine max FP 2 SV 1 381.64 0.8 (-0.22±0.38) [0.13] RBL_SOYBN Ribulose bisphosphate carboxylase large chain OS Glycine max GN rbcL PE 1 SV 3 675.63 0.8 (-0.22±0.58) [0.09] 03SPJ6_SOYBN Maturation protein PMX32 OS Glycine max GN PM32 PE 2 SV 1 135.96 0.8 (-0.22±0.46) [0.19] 10ST38_SOYBN Alcohol dehydrogenase Fragment OS Glycine max GN Adh 2 PE 3 SV 1 126.014 0.8 (-0.22±0.46) [0.19] 10SSEK8_SOYBN Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 3 SV 1 9770.59 0.83 (-0.19±0.45) [0.24] C6SZN7_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 7900.69 0.83 (-0.19±0.45) [0.24] C6SZN7_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 765.77 0.84 (-0.18±0.18) [0.02] C0EMU_SOYBN Dehydrin OS Glycine max GN PM24 PE 2 SV 1 8024.35 0.84 (-0.18±0.08) [0] C6SZN7_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 8024.35 0.84 (-0.18±0.08) [0] C6SWWA_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 8024.3	PARP3_SOYBN	Poly ADP ribose polymerase 3 OS Glycine max GN PARP3 PE 2 SV 1	316.88	0.79 (-0.24 ± 0.36) [0.1]
C6T605_S0YBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 381.64 0.8 (-0.22±0.38) [0.13] RBL_SOYBN Ribulose bisphosphate carboxylase large chain OS Glycine max GN rbcL PE 1 SV 3 675.63 0.8 (-0.22±0.38) [0.09] 09SPJ6_SOYBN Alcohol dehydrogenase Fragment OS Glycine max GN Adh 2 PE 3 SV 1 1260.14 0.8 (-0.22±0.46) [0.19] 09ST38_SOYBN Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2 18 789.71 0.81 (-0.21±0.42) [0.16] 09SEK8_SOYBN Seed maturation protein PM26 OS Glycine max GN PM26 PE 2 SV 1 970.59 0.83 (-0.19±0.18) [0.04] C6T8W5_SOYBN Putative uncharacterized protein DS Glycine max PE 2 SV 1 7690.69 0.83 (-0.19±0.41) [0.24] C6T8W5_SOYBN Putative uncharacterized protein DS Glycine max PE 2 SV 1 7690.69 0.83 (-0.19±0.41) [0.24] C6T8W5_SOYBN Putative uncharacterized protein DS Glycine max GN PM24 PE 2 SV 1 804 (-0.18±0.74) [0.28] OSELO_SOYBN Detative uncharacterized protein DS Glycine max PE 3 SV 1 28100.36 0.84 (-0.18±0.08) [0] C6SWW4_SOYBN Putative uncharacterized protein DS Glycine max PE 2 SV 1 804.41 (-0.18±0.08) [0] C6SWW4_SOYBN Putative uncharacterized protein DS Glycine max PE 2 SV 1 804.41 (-0.18±0.08) [0] <t< td=""><td>Q39801_SOYBN</td><td>51 kDa seed maturation protein OS Glycine max PE 2 SV 1</td><td>239.28</td><td>$0.79(-0.24\pm0.51)[0.19]$</td></t<>	Q39801_SOYBN	51 kDa seed maturation protein OS Glycine max PE 2 SV 1	239.28	$0.79(-0.24\pm0.51)[0.19]$
RBL_SOYBN Ribulose bisphosphate carboxylase large chain OS Glycine max GN rbcL PE 1 SV 3 675.63 0.8 (-0.22±0.33) [0.09] 09SPL6_SOYBN Maturation protein pPM32 OS Glycine max GN PM32 PE 2 SV 1 135.96 0.8 (-0.22±0.49) [0.29] 092T38_SOYBN Alcohol dehydrogenase Fragment OS Glycine max GN Adh 2 PE 3 SV 1 1260.14 0.8 (-0.22±0.46) [0.19] 1BBD2_SOYBN Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2 18789.71 0.81 (-0.21±0.42) [0.16] 09SEK8_SOYBN Seed maturation protein PM26 OS Glycine max PE 2 SV 1 9770.59 0.83 (-0.19±0.18] [0.04] 03BIW8_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 7690.69 0.83 (-0.19±0.45] [0.24] C6SZN7_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 760.57 0.84 (-0.18±0.74] [0.28] QSEL0_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 8024.35 0.84 (-0.18±0.74] [0.28] QSEVD_SOYBN Dehydrin OS Glycine max GN lea D 11 PE 3 SV 1 16 419.81 0.84 (-0.17±0.06] [0] C6TLT3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2810.36 0.84 (-0.17±0.06] [0] C6TLT3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 3	C6TG05_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	381.64	0.8 (-0.22 ± 0.38) [0.13]
D9SPJ6_SOYBN Maturation protein pPM32 OS Glycine max GN PM32 PE 2 SV 1 135.96 0.8 (-0.22 ± 0.59) (0.29) 0.92T38_SOYBN Alcohol dehydrogenase Fragment OS Glycine max GN Adh 2 PE 3 SV 1 1260.14 0.8 (-0.22 ± 0.46) (0.19) IBBD2_SOYBN Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2 18 789.71 0.81 (-0.21 ± 0.42) [0.16] 09SEK8_SOYBN Seed maturation protein PM26 OS Glycine max PE 2 SV 1 9770.59 0.83 (-0.19 ± 0.45) [0.24] C6T8W9_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2909.14 0.83 (-0.19 ± 0.45) [0.24] C6T8W9_SOYBN Putative uncharacterized protein OS Glycine max PE 4 SV 1 576.57 0.84 (-0.18 ± 0.14) [0.02] C6SZV7_SOYBN Putative uncharacterized protein OS Glycine max GN PM24 PE 2 SV 1 8024.35 0.84 (-0.18 ± 0.08) [0.22] C6SWVM4_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 8024.35 0.84 (-0.17 ± 0.06) [0] C6SWVM4_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 22 782.34 0.84 (-0.17 ± 0.05) [0] C6SWVM3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 28 100.36 0.84 (-0.14 ± 0.29) [0.19] C6SWV3_SOYBN Putative uncharacterized protein OS Glycine	RBL SOYBN	Ribulose bisphosphate carboxylase large chain OS Glycine max GN rbcL PE 1 SV 3	675.63	0.8 (-0.22+0.33) [0.09]
Q9ZT38_SOYBNAlcohol dehydrogenase Fragment OS Glycine max GN Adh 2 PE 3 SV 11260.14 $0.8 (-0.22 \pm 0.46) [0.19]$ IBBD2_SOYBNBowman Birk-type proteinase inhibitor D II OS Glycine max GN PM25 PE 2 SV 118 789.71 $0.81 (-0.21 \pm 0.42) [0.16]$ Q9SEK8_SOYBNSeed maturation protein PM26 OS Glycine max GN PM26 PE 2 SV 19770.59 $0.33 (-0.19 \pm 0.18) [0.04]$ Q38IW8_SOYBNTriosephosphate isomerase OS Glycine max PE 2 SV 12909.14 $0.83 (-0.19 \pm 0.18) [0.04]$ Q678W9_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 17690.69 $0.33 (-0.19 \pm 0.14) [0.02]$ C65ZN7_SOYBNPutative uncharacterized protein OS Glycine max GN PM24 PE 2 SV 18024.35 $0.84 (-0.18 \pm 0.18) [0.02]$ Q3SEL0_SOYBNDehydrin OS Glycine max GN lea D 11 PE 3 SV 116 419.81 $0.84 (-0.18 \pm 0.08) [0.02]$ C6SWW4_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 128100.36 $0.84 (-0.17 \pm 0.06) [0]$ B3TDK6_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 1284 40.17 \pm 0.06] [0]C6SWV3_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 12896.55 $0.87 (-0.14 \pm 0.29) [0.19]$ C6SWV3_SOYBNPutative uncharacterized protein OS Glycine max RE 2 SV 12896.55 $0.87 (-0.14 \pm 0.29) [0.19]$ Q9SKE2_SOYBNSeed maturation protein PM41 OS Glycine max RE 2 SV 12896.55 $0.87 (-0.14 \pm 0.29) [0.19]$ Q9SKE3_SOYBNSeed maturation protein PM41 OS Glycine max GN PM25 PE 2 SV 12842.41 $0.88 (-0.13 \pm 0.21) [0.21]$ Q3873_SOYBNLea protein OS Glycine max PE 2 SV 12846.50 $0.87 (-0.14 \pm 0$	Q9SPJ6 SOYBN	Maturation protein pPM32 OS Glycine max GN PM32 PE 2 SV 1	135.96	0.8 (-0.22+0.59) [0.29]
IBBD2_SOYBN Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2 18 789.71 0.81 (-0.21 ± 0.42) [0.16] Q3SEK8_SOYBN Seed maturation protein PM26 OS Glycine max PE 2 SV 1 9770.59 0.83 (-0.19 ± 0.18) [0.04] Q38IW8_SOYBN Triosephosphate isomerase OS Glycine max PE 2 SV 1 2909.14 0.83 (-0.19 ± 0.48) [0.24] C6T8W9_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 7690.69 0.83 (-0.19 ± 0.48) [0.24] C6SZN7_SOYBN Putative uncharacterized protein OS Glycine max PE 4 SV 1 576.57 0.84 (-0.18 ± 0.74) [0.28] QOSEL0_SOYBN Dehydrin OS Glycine max GN lea D 11 PE 3 SV 1 16 419.81 0.84 (-0.18 ± 0.18) [0.02] Q70EM0_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 28 100.36 0.84 (-0.17 ± 0.06) [0] G3TLK5_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 28 100.36 0.84 (-0.17 ± 0.05) [0] C6TLT3_SOYBN Putative uncharacterized protein OS Glycine max GN PM21 PE 2 SV 1 28 100.36 0.84 (-0.14 ± 0.29) [0.19] Q9SKE_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 28 100.36 0.84 (-0.17 ± 0.05) [0] C6TLT3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1	Q9ZT38 SOYBN	Alcohol dehydrogenase Fragment OS Glycine max GN Adh 2 PE 3 SV 1	1260.14	0.8 (-0.22+0.46) [0.19]
OSEKE_SOYBN Seed maturation protein PM26 OS Glycine max GN PM26 PE 2 SV 1 9770.59 0.83 (-0.19 ± 0.18) [0.04] Q38IW8_SOYBN Triosephosphate isomerase OS Glycine max PE 2 SV 1 2909.14 0.83 (-0.19 ± 0.15) [0.04] Q38IW8_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 7690.69 0.83 (-0.19 ± 0.14) [0.02] C65ZN7_SOYBN Putative uncharacterized protein OS Glycine max PE 4 SV 1 576.57 0.84 (-0.18 ± 0.14) [0.02] Q38L0_SOYBN Seed maturation protein PM24 OS Glycine max PE 4 SV 1 576.57 0.84 (-0.18 ± 0.14) [0.02] Q70EMO_SOYBN Dehydrin OS Glycine max GN PM24 PE 2 SV 1 8024.35 0.84 (-0.18 ± 0.08) [0] C65WW4_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 28 100.36 0.84 (-0.17 ± 0.06) [0] B3TDK6_SOYBN Lipoxygenase OS Glycine max PE 3 SV 1 22 782.34 0.84 (-0.17 ± 0.06) [0] C65WW4_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 3894.11 0.86 (-0.15 ± 0.21) [0.13] C65WW3_SOYBN Putative uncharacterized protein OS Glycine max GN PM25 PE 2 SV 1 3894.11 0.86 (-0.14 ± 0.13) [0.03] C65WW3_SOYBN Seed maturation protein PM25 OS Glycine max GN PM25 PE 2 SV 1 3964.62 <td>IBBD2 SOYBN</td> <td>Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2</td> <td>18 789.71</td> <td>0.81 (-0.21+0.42) [0.16]</td>	IBBD2 SOYBN	Bowman Birk-type proteinase inhibitor D II OS Glycine max PE 1 SV 2	18 789.71	0.81 (-0.21+0.42) [0.16]
Q38IW8_S0YBN Triosephosphate isomerase OS Glycine max PE 2 SV 1 2909.14 0.83 (-0.19 ± 0.45) [0.24] C678W9_S0YBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 7690.69 0.83 (-0.19 ± 0.14) [0.02] C65ZN7_S0YBN Putative uncharacterized protein OS Glycine max PE 4 SV 1 576.57 0.84 (-0.18 ± 0.74) [0.28] Q95EL_S0YBN Seed maturation protein PM24 OS Glycine max GN PM24 PE 2 SV 1 8024.35 0.84 (-0.18 ± 0.18] [0.02] Q70EM0_S0YBN Dehydrin OS Glycine max GN lea D 11 PE 3 SV 1 16 419.81 0.84 (-0.17 ± 0.06) [0] C6SWW4_S0YBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 28 100.36 0.84 (-0.17 ± 0.05) [0] C6TLT3_S0YBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 557.49 0.85 (-0.16 ± 0.81) [0.34] C6SWV3_S0YBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 3894.11 0.86 (-0.15 ± 0.21) [0.13] Q9SEK9_S0YBN Seed maturation protein PM41 OS Glycine max PE 2 SV 1 3894.11 0.86 (-0.14 ± 0.29) [0.19] Q9SEK9_S0YBN Seed maturation protein PM25 OS Glycine max GN PM25 PE 2 SV 1 9964.62 0.87 (-0.14 ± 0.23) [0.03] C6T7U2_S0YBN Lea protein OS Glycine max PE 2 SV 1 198.82 0.88	Q9SEK8 SOYBN	Seed maturation protein PM26 OS Glycine max GN PM26 PE 2 SV 1	9770.59	0.83(-0.19+0.18)[0.04]
C6T8W9_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 7690.69 0.83 (-0.19 ± 0.14) [0.02] C6SZN7_SOYBN Putative uncharacterized protein OS Glycine max GN PM24 PE 2 SV 1 576.57 0.84 (-0.18 ± 0.74) [0.28] Q9SEL0_SOYBN Seed maturation protein PM24 OS Glycine max GN PM24 PE 2 SV 1 8024.35 0.84 (-0.18 ± 0.74) [0.28] Q70EM0_SOYBN Dehydrin OS Glycine max GN lea D 11 PE 3 SV 1 16 419.81 0.84 (-0.18 ± 0.08) [0] C6SWW4_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 28 100.36 0.84 (-0.17 ± 0.06) [0] B3TDK6_SOYBN Lipoxygenase OS Glycine max PE 3 SV 1 22 782.34 0.84 (-0.17 ± 0.05) [0] C6TLT3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 557.49 0.85 (-0.16 ± 0.21) [0.13] Q9SWB2_SOYBN Putative uncharacterized protein OS Glycine max GN PM41 PE 4 SV 1 2896.55 0.87 (-0.14 ± 0.29) [0.19] Q9SEK9_SOYBN Seed maturation protein PM25 OS Glycine max GN PM25 PE 2 SV 1 9964.62 0.87 (-0.14 ± 0.13) [0.03] C6T7U2_SOYBN Putative uncharacterized protein OS Glycine max GN KTI2 PE 2 SV 1 3415.15 0.88 (-0.13 ± 0.21) [0.21] Q9SK85_SOYBN Seed maturation protein PM31 OS Glycine max GN KTI2 PE 2 SV 1	Q38IW8 SOYBN	Triosephosphate isomerase OS Glycine max PE 2 SV 1	2909.14	0.83(-0.19+0.45)[0.24]
C6SZN7_SOYBNPutative uncharacterized protein OS Glycine max PE 4 SV 1 576.57 084 (-0.18 ± 0.74) (0.28)Q9SEL0_SOYBNSeed maturation protein PM24 OS Glycine max GN PM24 PE 2 SV 1 8024.35 0.84 (-0.18 ± 0.74) (0.28)Q70EM0_SOYBNDehydrin OS Glycine max GN lea D 11 PE 3 SV 1 16419.81 0.84 (-0.18 ± 0.08) (0.02)Q70EM0_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 1 28100.36 0.84 (-0.17 ± 0.06) (0.02)C6SWW4_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 1 22782.34 0.84 (-0.17 ± 0.05) (0.03)C6TL7_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 1 557.49 0.85 (-0.16 ± 0.81) (0.34)C6SWV3_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 1 3894.11 0.86 (-0.15 ± 0.21) (0.13)Q9SWE2_SOYBNSeed maturation protein PM41 OS Glycine max GN PM41 PE 4 SV 1 2896.55 0.87 (-0.14 ± 0.29) (0.9)Q9SWE2_SOYBNSeed maturation protein PM25 OS Glycine max GN PM25 PE 2 SV 1 242.41 0.88 (-0.13 ± 0.31) (0.21)Q3873_SOYBNPutative uncharacterized protein OS Glycine max GN PM25 PE 2 SV 1 242.41 0.88 (-0.13 ± 0.31) (0.21)Q3873_SOYBNLea protein OS Glycine max PE 2 SV 1 21743.68 0.9 (-0.11 ± 0.07) (0.29)Q3873_SOYBNLipoxygenase OS Glycine max PE 3 SV 1 21743.68 0.9 (-0.11 ± 0.27) (0.29)Q3874_SOYBNLipoxygenase OS Glycine max PE 3 SV 1 21743.68 0.9 (-0.11 ± 0.07) (0.29 Q3875_SOYBNLipoxygenase OS Glycine max PE 3 SV 1 2284.28	C6T8W9 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	7690.69	0.83(-0.19+0.14)[0.02]
OBSEL_SOYBN Seed maturation protein PM24 OS Glycine max GN PM24 PE 2 SV 1 8024.35 0.84 (-0.18 ± 0.18) [0.02] Q70EM0_SOYBN Dehydrin OS Glycine max GN lea D 11 PE 3 SV 1 16 419.81 0.84 (-0.18 ± 0.18) [0.02] Q70EM0_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 28 100.36 0.84 (-0.18 ± 0.18) [0.02] G6SWW4_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 22 782.34 0.84 (-0.17 ± 0.05) [0] C6TLT3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 557.49 0.85 (-0.16 ± 0.81) [0.34] C6SWV3_SOYBN Putative uncharacterized protein OS Glycine max GN PM41 PE 4 SV 1 2896.55 0.87 (-0.14 ± 0.29) [0.19] Q9SEL9_SOYBN Seed maturation protein PM41 OS Glycine max GN PM25 PE 2 SV 1 9964.62 0.87 (-0.14 ± 0.13) [0.03] C6T7U2_SOYBN Putative uncharacterized protein OS Glycine max GN PM25 PE 2 SV 1 242.41 0.88 (-0.13 ± 0.31) [0.21] Q3873_SOYBN Lea protein OS Glycine max PE 3 SV 1 242.41 0.88 (-0.13 ± 0.52) [0.31] KT12_SOYBN Kunitz-type trypsin inhibitor KT12 OS Glycine max GN KT12 PE 2 SV 1 3415.15 0.89 (-0.12 ± 0.25) [0.24] B3TDK5_SOYBN Liap oxtenacterized protein OS Glycine max GN PM3	C6SZN7 SOYBN	Putative uncharacterized protein OS Glycine max PE 4 SV 1	576.57	0.84 (-0.18 + 0.74) [0.28]
D70EM0_S0YBNDehydrin OS Glycine max GN lea D 11 PE 3 SV 116 419.81 $0.84 (-0.18 \pm 0.08)$ [0]C6SWW4_S0YBNPutative uncharacterized protein OS Glycine max PE 2 SV 128 100.36 $0.84 (-0.17 \pm 0.06)$ [0]B3TDK6_S0YBNLipoxygenase OS Glycine max PE 3 SV 122 782.34 $0.84 (-0.17 \pm 0.05)$ [0]C6TLT3_S0YBNPutative uncharacterized protein OS Glycine max PE 2 SV 1557.49 $0.85 (-0.16 \pm 0.81)$ [0.34]C6SWV3_S0YBNPutative uncharacterized protein OS Glycine max PE 2 SV 13894.11 $0.86 (-0.15 \pm 0.21)$ [0.13]Q9SWB2_S0YBNSeed maturation protein PM41 OS Glycine max GN PM41 PE 4 SV 12896.55 $0.87 (-0.14 \pm 0.29)$ [0.19]Q9SEK9_S0YBNSeed maturation protein PM25 OS Glycine max GN PM25 PE 2 SV 19964.62 $0.87 (-0.14 \pm 0.13)$ [0.03]C6T7U2_S0YBNPutative uncharacterized protein OS Glycine max GN FM25 PE 2 SV 1242.41 $0.88 (-0.13 \pm 0.31)$ [0.21]Q39873_S0YBNLea protein OS Glycine max PE 2 SV 1198.82 $0.88 (-0.13 \pm 0.52)$ [0.31]KT12_S0YBNKunitz-type trypsin inhibitor KT12 OS Glycine max GN KT12 PE 2 SV 13415.15 $0.89 (-0.12 \pm 0.25)$ [0.24]B3TDK5_S0YBNLipoxygenase OS Glycine max PE 3 SV 121743.68 $0.9 (-0.11 \pm 0.07)$ [0]Q9XET1_S0YBNPutative uncharacterized protein OS Glycine max PE 2 SV 12324.38 $0.9 (-0.11 \pm 0.07)$ [0]Q9XET1_S0YBNLipoxygenase OS Glycine max PE 3 SV 123 234.38 $0.9 (-0.11 \pm 0.45)$ [0.37]B3TDK4_S0YBNLipoxygenase OS Glycine max GN VM2 PE 2 SV 123 234.38 $0.9 (-0.1 \pm 0.45)$ [0.37]C6TMK3_S0YBNLipoxygenase OS	Q9SEL0_SOYBN	Seed maturation protein PM24 OS Glycine max GN PM24 PE 2 SV 1	8024.35	$0.84 (-0.18 \pm 0.18) [0.02]$
C6SWW4_S0YBNPutative uncharacterized protein OS Glycine max PE 2 SV 128 100.36 $0.84 (-0.17 \pm 0.06) [0]$ B3TDK6_S0YBNLipoxygenase OS Glycine max PE 3 SV 122 782.34 $0.84 (-0.17 \pm 0.06) [0]$ C6TLT3_S0YBNPutative uncharacterized protein OS Glycine max PE 2 SV 1 557.49 $0.85 (-0.16 \pm 0.81) [0.34]$ C6SWW3_S0YBNPutative uncharacterized protein OS Glycine max PE 2 SV 1 3894.11 $0.86 (-0.15 \pm 0.21) [0.13]$ Q9SWB2_S0YBNSeed maturation protein PM41 OS Glycine max GN PM41 PE 4 SV 1 2896.55 $0.87 (-0.14 \pm 0.29) [0.19]$ Q9SEK9_S0YBNSeed maturation protein PM25 OS Glycine max GN PM25 PE 2 SV 1 9964.62 $0.87 (-0.14 \pm 0.13) [0.03]$ C6T7U2_S0YBNPutative uncharacterized protein OS Glycine max GN PM25 PE 2 SV 1 9964.62 $0.87 (-0.14 \pm 0.13) [0.21]$ Q39873_S0YBNLea protein OS Glycine max PE 2 SV 1 198.82 $0.88 (-0.13 \pm 0.52) [0.31]$ XT12_S0YBNLea protein OS Glycine max PE 3 SV 1 242.41 $0.88 (-0.13 \pm 0.52) [0.24]$ B3TDK5_S0YBNLipoxygenase OS Glycine max PE 3 SV 1 21743.68 $0.9 (-0.11 \pm 0.07) [0]$ Q9XET1_S0YBNLipoxygenase OS Glycine max PE 3 SV 1 21743.68 $0.9 (-0.11 \pm 0.07) [0]$ Q9XET1_S0YBNLipoxygenase OS Glycine max PE 3 SV 1 223234.38 $0.9 (-0.11 \pm 0.03) [0.37]$ G6ST1_S0YBNLipoxygenase OS Glycine max PE 3 SV 1 223234.38 $0.9 (-0.11 \pm 0.03) [0.37]$ G6ST1_S0YBNLipoxygenase OS Glycine max PE 3 SV 1 23234.38 $0.9 (-0.1 \pm 0.08) [0.31]$ G6ST1_S0YBNLipoxygenase OS Glycine max GN Vix C PE 2 SV 1 707	Q70EM0 SOYBN	Dehydrin OS Glycine max GN lea D 11 PE 3 SV 1	16 419.81	0.84(-0.18+0.08)[0]
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DescriptionControl </td <td>C6SWV3_SOYBN</td> <td>Putative uncharacterized protein OS Glycine max PE 2 SV 1</td> <td>3894.11</td> <td>0.86(-0.15+0.21)[0.13]</td>	C6SWV3_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	3894.11	0.86(-0.15+0.21)[0.13]
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Cathologic DistributionDistributionDistributionDistributionC6T7U2_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 1242.41 $0.88 (-0.13 \pm 0.31) [0.21]$ C39873_SOYBNLea protein OS Glycine max PE 2 SV 1198.82 $0.88 (-0.13 \pm 0.52) [0.31]$ KTI2_SOYBNKunitz-type trypsin inhibitor KTI2 OS Glycine max GN KTI2 PE 2 SV 13415.15 $0.89 (-0.12 \pm 0.25) [0.24]$ B3TDK5_SOYBNLipoxygenase OS Glycine max PE 3 SV 121743.68 $0.9 (-0.11 \pm 0.07) [0]$ Q9XET1_SOYBNSeed maturation protein PM31 OS Glycine max GN PM31 PE 2 SV 19168.43 $0.9 (-0.11 \pm 0.13) [0.07]$ C6SZ11_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 12284.28 $0.9 (-0.11 \pm 0.45) [0.37]$ B3TDK4_SOYBNLipoxygenase OS Glycine max FE 3 SV 123 234.38 $0.9 (-0.1 \pm 0.08) [0.01]$ Q43440_SOYBNLipoxygenase OS Glycine max GN vkC PE 2 SV 17075.76 $0.91 (-0.09 \pm 0.5) [0.35]$ C6TMK3_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 1202.81 $0.92 (-0.08 \pm 1.16) [0.45]$ C0XX_SOVBNSeed lipoxygenase OS Glycine max GN VLC PE 2 SV 1 $202.81 0.92 (-0.08 \pm 1.16) [0.45]$	09SFK9_SOYBN	Seed maturation protein PM25 OS Glycine max GN PM25 PE 2 SV 1	9964.62	0.87 (-0.14 + 0.13) [0.03]
ConstrainedLitterConstrained <td>C6T7U2_SOYBN</td> <td>Putative uncharacterized protein OS Glycine max PE 2 SV 1</td> <td>242 41</td> <td>$0.88 (-0.13 \pm 0.31) [0.21]$</td>	C6T7U2_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	242 41	$0.88 (-0.13 \pm 0.31) [0.21]$
ABSOF0_COTB_N Lidd protein of or of with max FE 2 of 1 10032 0.00 (-0.12 ± 0.25) [0.24] KTI2_SOYBN Kunitz-type tryps in inhibitor KTI2 OS Glycine max GN KTI2 PE 2 SV 1 3415.15 0.89 (-0.12 ± 0.25) [0.24] B3TDK5_SOYBN Lipoxygenase OS Glycine max PE 3 SV 1 21 743.68 0.9 (-0.11 ± 0.07) [0] Q9XET1_SOYBN Seed maturation protein PM31 OS Glycine max GN PM31 PE 2 SV 1 9168.43 0.9 (-0.11 ± 0.13) [0.07] C6SZ11_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2284.28 0.9 (-0.11 ± 0.45) [0.37] B3TDK4_SOYBN Lipoxygenase OS Glycine max PE 3 SV 1 23 234.38 0.9 (-0.1 ± 0.08) [0.01] Q43440_SOYBN Lipoxygenase OS Glycine max GN vlxC PE 2 SV 1 7075.76 0.91 (-0.09 ± 0.5) [0.35] C6TMK3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 202.81 0.92 (-0.08 ± 1.16) [0.45] QAX SOYBN Seed lipoxygenase OS Glycine max GN vlxC PE 2 SV 1 202.81 0.92 (-0.08 ± 1.16) [0.45] QAX SOYBN Seed lipoxygenase OS Glycine max GN LOX1 4 PE 1 SV 1 6111 7 0.92 (-0.08 ± 1.16) [0.45]	039873 SOVEN	Lea protein OS Glycine max PE 2 SV 1	198.82	$0.88(-0.13\pm0.52)[0.31]$
RATE_CONSIGN Lipoxygenase OS Glycine max PE 3 SV 1 21 743.68 0.9 (-0.11±0.17) [0] Q9XET1_SOYBN Seed maturation protein PM31 OS Glycine max GN PM31 PE 2 SV 1 9168.43 0.9 (-0.11±0.13) [0.07] C6SZ11_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 21 743.68 0.9 (-0.11±0.13) [0.07] C6SZ11_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 2284.28 0.9 (-0.11±0.45) [0.37] B3TDK4_SOYBN Lipoxygenase OS Glycine max PE 3 SV 1 23 234.38 0.9 (-0.1±0.08) [0.01] Q43440_SOYBN Lipoxygenase OS Glycine max GN vkC PE 2 SV 1 7075.76 0.91 (-0.09±0.5) [0.35] C6TMK3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 202.81 0.92 (-0.03±0.5) [0.45] LOXX SOYBN Seed lipoxygenase OS Glycine max GN 10X1 4 PE 1 SV 1 6111.07 0.92 (-0.07±0.34) [0.41]	KTI2 SOYBN	Kunitz-tyne trynsin inhibitor KTI2 OS Glycine max GN KTI2 PE 2 SV 1	3415.15	$0.89(-0.12\pm0.25)[0.24]$
DescriptionDescriptionDescriptionDescriptionDescriptionQ9XET1_SOYBNSeed maturation protein PM31 OS Glycine max GN PM31 PE 2 SV 19168.430.9 (-0.11 ± 0.13) [0.07]C6SZ11_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 12284.280.9 (-0.11 ± 0.45) [0.37]B3TDK4_SOYBNLipoxygenase OS Glycine max PE 3 SV 123 234.380.9 (-0.1 ± 0.08) [0.01]Q43440_SOYBNLipoxygenase OS Glycine max GN vlxC PE 2 SV 17075.760.91 (-0.09 ± 0.5) [0.35]C6TMK3_SOYBNPutative uncharacterized protein OS Glycine max PE 2 SV 1202.810.92 (-0.08 ± 1.16) [0.45]QXX_SOYBNSeed lipoxygenase OS Glycine max GN LOX1 4 PE 1 SV 15111 070.92 (-0.08 ± 0.34) [0.41]	B3TDK5_SOYBN	Linoxygenase OS Glycine max PE 3 SV 1	21 743 68	0.9(-0.11+0.07)[0]
C6SZ11_SOYBN Putative uncharacterized protein OS Glycine max GN PMBTP 2 SV 1 2284.28 0.9 (-0.1±0.45) [0.37] B3TDK4_SOYBN Lipoxygenase OS Glycine max PE 2 SV 1 23 234.38 0.9 (-0.1±0.08) [0.01] Q43440_SOYBN Lipoxygenase OS Glycine max PE 2 SV 1 23 234.38 0.9 (-0.1±0.08) [0.01] C6TMK3_SOYBN Lipoxygenase OS Glycine max GN vlxC PE 2 SV 1 7075.76 0.91 (-0.09±0.5) [0.35] C6TMK3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 202.81 0.92 (-0.08±1.16) [0.45] OXX SOYBN Seed lipoxygenase OS Glycine max GN LOX1 4 PE 1 SV 1 5111 07 0.92 (-0.07±0.34) [0.41]	09XET1_SOVBN	Seed maturation protein PM31 OS Glycine max GN PM31 PE 2 SV 1	9168 43	$0.0 (-0.11 \pm 0.07) [0]$
B3TDK4_SOYBN Lipoxygenase OS Glycine max PE 3 SV 1 23 234.38 $0.9 (-0.1 \pm 0.08) [0.01]$ Q43440_SOYBN Lipoxygenase OS Glycine max GN vlxC PE 2 SV 1 7075.76 $0.91 (-0.09 \pm 0.5) [0.35]$ C6TMK3_SOYBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 202.81 $0.92 (-0.08 \pm 1.16) [0.45]$ LOXX_SOYBN Seed lipoxygenase OS Glycine max GN LOX1 4 PE 1 SV 1 6111 07 $0.92 (-0.07 \pm 0.34) [0.41]$	C6SZ11_SOVEN	Putative uncharacterized protein OS Glycine may PE 2 SV 1	2284.28	$0.9(-0.11 \pm 0.45)[0.37]$
Construction	B3TDK4 SOVEN	Linoxygenase OS Glycine max PF 3 SV 1	2204.20	0.9(-0.1+0.08)[0.01]
C6TMK3_S0YBN Putative uncharacterized protein OS Glycine max PE 2 SV 1 10XX S0YBN Seed lipoxygenase OS Glycine max GN L0X1 4 PE 1 SV 1 10XX S0YBN SE S0	NA3440 SOVEN	Lipoxygenase OS Glycine max FL 3 50 F	20 204.00	0.01 (-0.02 + 0.00) [0.01]
$\frac{10000}{1000} = 100000 = 100000 = 100000 = 100000 = 100000 = 100000 = 100000 = 100000 = 100000 = 100000 = 100000 = 100000 = 100000 = 100000 = 100000000$	C6TMK3 SOVRN	Putative uncharacterized protein OS Glycine may PF 2 SV 1	202.81	0.01 (-0.03 + 0.07 + 0.05)
	LOXX SOVEN	Seed linoxygenase OS Glycine max GN LOX1 4 PF 1 SV 1	6111.07	0.93(-0.07+0.34)[0.41]

Table 3. Continued

Accession	Description	Score	Soy Cn: Soy CTAG L3
C6TKH0 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	25 943 07	0.93 (-0.07 + 0.08) [0.04]
C6SZK3 SOYBN	Glutathione peroxidase OS Glycine max PE 2 SV 1	737.65	0.93 (-0.07 + 0.72) [0.46]
0587K1 SOYBN	BiP OS Glycine max GN Gm bin PE 2 SV 1	744.24	0.94 (-0.06 + 0.43) [0.43]
C6T0B5 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	2535.7	0.94 (-0.06 + 0.22) [0.25]
C6TB98 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	664.43	0.95 (-0.05 + 0.49) [0.41]
A7LCD5 SOYBN	Lipoxygenase OS Glycine max GN LOX9 PE 2 SV 1	791.73	0.95(-0.05+0.34)[0.45]
C6TBB3_SOYBN	Putative uncharacterized protein OS Glycine max PE 4 SV 1	4899.04	$0.95 (-0.05 \pm 0.69) [0.44]$
09XES8 SOYBN	Seed maturation protein PM28 OS Glycine max GN PM28 PE 4 SV 1	3193.13	0.96(-0.04+0.25)[0.35]
C6T1Q7 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	7730	0.96(-0.04+0.13)[0.26]
C6SVM2_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	4510.81	$0.96 (-0.04 \pm 0.24) [0.39]$
Q948Y0_SOYBN	β -Conglycinin α prime subunit OS Glycine max PE 2 SV 1	23 517.56	0.96 (-0.04 ± 0.06) [0.13]
C6T9C2_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	842.05	$0.98 (-0.02 \pm 0.2) [0.44]$
HSP70_SOYBN	Heat shock 70 kDa protein OS Glycine max GN HSP70 PE 3 SV 1	1481.25	1 (0±0.19) [0.44]
C6TGA6_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	227.22	1 (0±0.93) [0.49]
Q9XET0_SOYBN	Putative uncharacterized protein OS Glycine max GN PM30 PE 2 SV 1	5145.05	1 (0±0.15) [0.55]
C6T1V2 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	727.8	1.01 (0.01 + 0.44) [0.54]
C6TAA6 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	1169.44	1.02 (0.02+0.63) [0.49]
C6SYU0 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	422.08	1.05(0.05+0.37)[0.64]
C6K8D1 SOYBN	Seed biotinylated protein 68 kDa isoform OS Glycine max PE 2 SV 1	782	1.05 (0.05+0.19) [0.69]
B102X4 SOYBN	Protein disulfide isomerase OS Glycine max GN PDIL 1 PE 3 SV 1	190.14	1.06 (0.06 + 0.38) [0.58]
C6TNU2 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	684.36	1.07 (0.07 + 1.03) [0.6]
GLYG1 SOYBN	Glycinin G1 OS Glycine max GN GY1 PE 1 SV 2	34 638.61	1.07 (0.07 + 0.07) [0.98]
Q6RIB7 SOYBN	Enolase OS Glycine max PE 2 SV 1	518.48	1.09 (0.09+0.29) [0.68]
C6T588 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	7076.11	1.13 (0.12+0.17) [0.92]
C6TCR6 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	163.95	1.13 (0.12+0.47) [0.65]
C6T9Z5 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	11 699.31	1.15 (0.14+0.12) [0.99]
LEC SOYBN	Lectin OS Glycine max GN LE1 PE 1 SV 1	11 981.52	1.16 (0.15+0.06) [1]
C6TB70 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	3485.42	1.16 (0.15+0.19) [0.89]
C6TK76 SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	1249.91	1.16 (0.15+0.72) [0.62]
C6TKJ5_SOYBN	Triosephosphate isomerase OS Glycine max PE 2 SV 1	2573.78	1.19 (0.17±0.26) [0.84]
C6TB67_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	1790.65	1.25 (0.22 ± 0.26) [0.92]
Q9ARI1_SOYBN	Lipoxygenase Fragment OS Glycine max PE 2 SV 1	5806.59	1.27 (0.24±0.59) [0.76]
C6SXU0_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	295.31	1.27 (0.24 ± 0.38) [0.81]
Q7XAC5_SOYBN	Embryo-specific urease OS Glycine max PE 2 SV 1	128.19	1.27 (0.24 ± 0.49) [0.84]
Q9XER5_SOYBN	Seed maturation protein PM22 OS Glycine max GN PM22 PE 2 SV 1	7963.38	1.3 (0.26±0.13) [1]
C6TNT2_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	680.48	1.35 (0.3±0.97) [0.78]
P93165_SOYBN	Em protein OS Glycine max PE 4 SV 1	4895.4	1.36 (0.31±0.5) [0.91]
022121_S0YBN	β -Subunit of β conglycinin Fragment OS Glycine max PE 2 SV 2	26 903.96	1.36 (0.31±0.04) [1]
C6T8Q2_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	1063.38	1.39 (0.33±1.17) [0.8]
064458_SOYBN	Gly m Bd 30K allergen OS Glycine max GN P34 PE 2 SV 1	2154.71	1.46 (0.38±0.2) [1]
C6EVF9_SOYBN	Elongation factor 1 α OS Glycine max GN EF 1A PE 2 SV 1	1653.6	1.49 (0.4±0.16) [1]
C6TCF1_SOYBN	Putative uncharacterized protein OS Glycine max PE 2 SV 1	2055.68	1.62 (0.48±0.75) [0.94]
LOX3_SOYBN	Seed lipoxygenase 3 OS Glycine max GN LOX1 3 PE 1 SV 1	21 758.1	1.75 (0.56±0.12) [1]
Q96444_SOYBN	Actin Fragment OS Glycine max GN Soy119 PE 3 SV 1	662.59	1.75 (0.56±1.42) [0.84]
Q39816_SOYBN	7S storage protein α subunit OS Glycine max PE 2 SV 1	11 664.26	1.82 (0.6±0.18) [1]
Q50JD8_SOYBN	eta-Conglycinin eta subunit Fragment OS Glycine max PE 2 SV 1	26 292.45	1.84 (0.61±0.11) [1]
SBP_SOYBN	Sucrose-binding protein OS Glycine max GN SBP PE 1 SV 1	4608.48	1.93 (0.66±0.1) [1]
Q8GV24_SOYBN	Nucleoside diphosphate kinase OS Glycine max PE 2 SV 1	442.06	1.95 (0.67 ± 1.16) [0.88]
Q948X9_SOYBN	eta-Conglycinin $lpha$ subunit OS Glycine max PE 2 SV 1	18 794	2.08 (0.73±0.06) [1]
Q96442_SOYBN	Actin Fragment OS Glycine max GN Soy57 PE 3 SV 1	1118.66	2.1 (0.74 <u>+</u> 1.37) [0.88]
A7LCD6_SOYBN	Lipoxygenase OS Glycine max GN LOX10 PE 2 SV 1	419.03	2.12 (0.75±0.39) [1]
Q7GC77_SOYBN	Glycinin A3B4 subunit OS Glycine max PE 1 SV 1	14 016.68	2.2 (0.79±0.35) [1]
Q9SB11_SOYBN	Glycinin OS Glycine max GN A5A4B3 PE 2 SV 1	13 858.51	2.2 (0.79±0.05) [1]
Q9SP11_SOYBN	Sucrose-binding protein homolog S 64 OS Glycine max GN SBP PE 2 SV 1	1005.86	2.29 (0.83±0.44) [1]
GLYG4_SOYBN	Glycinin G4 OS Glycine max GN GY4 PE 1 SV 1	13 834.81	2.41 (0.88±0.07) [1]
004299_SOYBN	Elongation factor 1 α Fragment OS Glycine max GN TefS1 PE 4 SV 1	957.33	2.59 (0.95±0.68) [1]
U6V7V6_SOYBN	34 kDa maturing seed vacuolar thiol protease Fragment OS Glycine max PE 2 SV 1	1671.61	2.97 (1.09±0.76) [0.97]
B3TDK9_SOYBN	Lipoxygenase US Glycine max PE 3 SV 1	1434.12	3 (1.1±0.2) [1]
GLYG2_SUYBN	Giycinin G2 US Giycine max GN Gy2 PE 1 SV 2	26 155.1	3.46 (1.24±0.05) [1]

Tab	e 3.	Continued
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Accession	Description	Score	Soy Cn: Soy CTAG L3
HPSE_SOYBN	Hydrophobic seed protein OS Glycine max PE 1 SV 1	17 254.79	3.53 (1.26±0.63) [0.99]
7SB1_SOYBN	Basic 7S globulin OS Glycine max GN BG PE 1 SV 2	192.05	6.69 (1.9±0.29) [1]
Q43446_SOYBN	Lipoxygenase OS Glycine max GN vlxB PE 1 SV 1	2417.81	7.61 (2.03±0.52) [1]
Q39874_SOYBN	Lipoxygenase Fragment OS Glycine max PE 2 SV 1	10 427.04	12.81 (2.55±0.33) [1]
ACT1_SOYBN	Actin 1 OS Glycine max GN SAC1 PE 3 SV 2	1152.62	21.54 (3.07 ± 0.89) [1]
GLYG5_SOYBN	Glycinin OS Glycine max PE 1 SV 1	8659.8	26.84 (3.29±0.23) [1]
Q39858_SOYBN	Soybean glycinin A3 B4 subunit Fragment OS Glycine max PE 2 SV 1	7988.31	30.27 (3.41±0.29) [1]
Q852U4_SOYBN	Glycinin A1bB2 784 OS Glycine max PE 2 SV 1	12 395.54	49.4 (3.9±0.41) [1]

reproducibility between different injections, usually in the full loop method, with 2 μL or 5 μL sample injection loading.

Figure 2 shows the resulting nanoUPLC chromatogram, the MS^E spectra from the $[M+2H]^{+2} = 857.87$ CTAG fragment and the respective spectra processed using PLGS. Five peptides from CTAG (Table 1, Fig. 1) were detected with high selectivity and specificity. These peptides showed no trace of post-translational modification; however, that possibility cannot be discarded because an additional 6 CTAG peptides were not detected. The orthogonal separations [48] obtained using SCX columns [49, 50] or recent technologies at the first-dimension linear gradient with fractions at different pH levels and with high-resolution separations in both the first and the second dimensions [51] were permitted because of the complexity of the chromatogram in this particular sample (Fig. 2A). Other methods for protein expression, including label-free with a continuous ion current and parallel fragmentation, have been used extensively for the monitoring of protein expression and have been described elsewhere [52].

3.2 Expression analysis

Additionally, a proteomic profile was processed with absolute quantitative values for each protein (Table 2). The CTAG recombinant protein was detected and quantified in nanograms based on the stoichiometric ion intensity values of the minimum three prototypic peptides of ADH and the identified protein. A relationship between the total detected protein and the specific protein concentration was determined, which allowed for the calculation of the percentage of the expressed protein in relation to TSP. The percentages of each detected protein are listed in Table 2. CTAG had an expression value of 0.1%, which is low compared with that of the other transgenic soybean seeds that express hGH [9] (2.9%), but it had a similar value compared with factor IX expression (0.2%) [10]. Other soybean proteins, such as β-conglycinin and glycinin, have been shown to exhibit the expected values that would be predicted for these storage proteins in soybean seeds [53].

Using this method, it was also possible to check protein expression regulation by comparing two or more samples.

Table 3 shows a two-by-two comparison of the Soy CTAG L3 and Soy Cn protein expression list. This technique can also be used to check higher and lower regulations of native proteins, which are similar to the functions of the Identity^E and Expression^E software in PLGS (Waters) and provide information regarding the side effects at the proteomic level of the introduction of transgenes. In this case, a maturation protein, p24 oleosin, and a β conglycinin fragment were down-regulated in the Soy CTAG L3 transgenic line (Table 3). However, upregulations of glycinin, lectin and actin 1 were observed in the transgenic line (Table 3). This information extends the range of the technique and helps confirm that CTAG was expressed only in the Soy CTAG L3 sample.

4 Concluding remarks

This is an easy-to-follow method of determining whether a target recombinant protein is expressed in any expression system, especially in a situation where a small sample must be used or no antibody is available to perform blotting detection methods. Two samples, Soy CTAG L3 and Soy Cnnegative from the BR-16 cultivar, were used. The results indicate that Soy CTAG L3 was present in the sample and represented 0.1% of the TSP sample, which was low compared with the yields of the other recombinant proteins that have been expressed in soybean. The proteomic profile was also processed with absolute quantitation for each identified protein, which enabled the identification and analysis of higher and lower regulations of native proteins.

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5 References

- [1] Swartz, J. R., Curr. Opin. Biotechnol. 2001, 12, 195–201.
- [2] Chu, L., Robinson, D. K., Curr. Opin. Biotechnol. 2001, 12, 180–187.
- [3] Tremblay, R., Wang, D., Jevnikar, A. M., Ma, S., Biotechnol. Adv. 2010, 28, 214–221.
- [4] Daniell, H., Singh, N. D., Mason, H., Streatfield, S. J., *Trends Plant Sci.* 2009, 14, 669–679.
- [5] Boothe, J., Nykiforuk, C., Shen, Y., Zaplachinski, S., Szarka, S., Kuhlman, P., Murray, E., Morck, D., Moloney, M. M., *Plant Biotechnol. J.* 2010, *8*, 588–606.
- [6] Cunha, N. B. d., Araújo, A. C. G., Leite, A., Murad, A. M., Vianna, G. R., Rech, E. L., *Genet. Mol. Res.* 2010, *9*, 1163–1170.
- [7] Jolliffe, N. A., Craddock, C. P., Frigerio, L., Biochem. Soc. Trans. 2005, 33, 1016–1018.
- [8] Ma, J. K.-C., Drake, P. M. W., Christou, P., Nat. Rev. Genet. 2003, 4, 794–805.
- [9] Cunha, N. B., Murad, A. M., Cipriano, T. M., Araujo, A. C. G., Aragao, F. J. L., Leite, A., Vianna, G. R., McPhee, T. R., Souza, G. H. M. F., Waters, M. J., Rech, E. L., *Transgenic Res.* 2011, *20*, 811–826.
- [10] Cunha, N. B., Murad, A. M., Ramos, G. L., Maranhao, A. Q., Brỳgido, M. M., Araujo, A. C. G., Lacorte, C., Aragao, F. J. L., Covas, D. T., Fontes, A. M., Souza, G. H. M. F., Vianna, G. R., Rech, E. L., *Transgenic Res.* 2011, *20*, 841–855.
- [11] Rech, E. L., Vianna, G. R., Aragão, F. J. L., *Nat. Protoc.* 2008, *3*, 410–418.
- [12] Blas, A. L. D., Cherwinski, H. M., Anal. Biochem. 1983, 133, 214–219.
- [13] Perlmann, P., Engvall, E., Immunochemistry 1971, 8, 871–874.
- [14] O'Farrells, P. H., J. Biol. Chem. 1975, 250, 4007-4021.
- [15] Shevchenko, A., Tomas, H., Havlis, J., Olsen, J. V., Mann, M., *Nat. Protoc.* 2006, *1*, 2856–2860.
- [16] Weiss, W., Görg, A., Methods Mol. Biol. 2007, 355, 121–143.
- [17] Blackstock, W. P., Weir, M. P., *Trends Biotechnol.* 1999, 17, 121–127.
- [18] Murad, A. M., Laumann, R. A., Lima, T. d. A., Sarmento, R. B. C., Noronha, E. F., Rocha, T. L., Valadares-Inglis, M. C., Franco, O. L., *Comp. Biochem. Physiol. C* 2006, *142*, 365–370.
- [19] Murad, A. M., Noronha, E. F., Miller, R. N. G., Costa, F. T., Pereira, C. D., Mehta, Â., Caldas, R. A., Franco, O. L., *Microbiology* 2008, *154*, 3766–3774.
- [20] Halligan, B. D., Methods Mol. Biol. 2009, 527, 283–298.
- [21] Henzel, W. J., Billeci, T. M., Stults, J. T., Wong, S. C., Grimley, C., Watanabe, C., *Proc. Natl. Acad. Sci. USA* 1993, *90*, 5011–5015.
- [22] Wilson, N., Simpson, R., Cooper-Liddell, C., *Methods Mol. Biol.* 2009, *534*, 205–212.
- [23] Gevaert, K., Goethals, M., Martens, L., Damme, J. V., Staes, A., Thomas, G. R., Vandekerckhove, J., Nat. Biotechnol. 2003, 21, 566–569.

- [24] Hunter, A. P., Games, D. E., *Rapid Commun. Mass. Spectrom.* 1994, *8*, 559–570.
- [25] Wilkins, J. A., Xiang, R., Horváth, C., Anal. Chem. 2002, 74, 3933–3941.
- [26] Guerrier, L., Righetti, P. G., Boschetti, E., Nat. Protoc. 2008, 3, 883–890.
- [27] Shen, Y., Zhao, R., Berger, S. J., Anderson, G. A., Rodriguez, N., Smith, R. D., *Anal. Chem.* 2002, 74, 4235–4249.
- [28] Nouri, M.-Z., Komatsu, S., Proteomics 2010, 10, 1930–1945.
- [29] Brumbarova, T., Matros, A., Mock, H.-P., Bauer, P., *Plant J.* 2008, *54*, 321–334.
- [30] Behrens, H. L., Chen, R., Li, L., Anal. Chem. 2008, 80, 6949–6958.
- [31] Zybailov, B., Rutschow, H., Friso, G., Rudella, A., Emanuelsson, O., Sun, Q., Wijk, K. J. v., *PLoS One* 2008, *3*, e1994.
- [32] Barnes, S., Shonsey, E. M., Eliuk, S. M., Stella, D., Barrett, K., Srivastava, O. P., Kim, H., Renfrow, M. B., Biochem. Soc. Trans. 2008, 36, 1037–1044.
- [33] Bouché, J.-P., Froment, C., Dozier, C., Esmenjaud-Mailhat, C., Lemaire, M., Monsarrat, B., Burlet-Schiltz, O., Ducommun, B., *J. Proteome Res.* 2008, 7, 1264–1273.
- [34] Unwin, R. D., Griffiths, J. R., Whetton, A. D., *Nat. Protoc.* 2009, *4*, 870–877.
- [35] Mori, M., Abe, K., Yamaguchi, H., Goto, J., Shimada, M., Mano, N., J. Proteome Res. 2010, 9, 3741–3749.
- [36] Yang, Y., Zhang, S., Howe, K., Wilson, D. B., Moser, F., Irwin, D., Thannhauser, T. W., *J. Biomol. Tech.* 2007, *18*, 226–237.
- [37] Levin, Y., Wang, L., Ingudomnukul, E., Schwarz, E., Baron-Cohen, S., Palotás, A., Bahn, S., *J. Chromatogr. B* 2009, *877*, 1299–1305.
- [38] Li, G.-Z., Vissers, J. P. C., Silva, J. C., Golick, D., Gorenstein, M. V., Geromanos, S. J., *Proteomics* 2009, *9*, 1696–1719.
- [39] Geromanos, S. J., Vissers, J. P. C., Silva, J. C., Dorschel, C. A., Li, G.-Z., Gorenstein, M. V., Bateman, R. H., Langridge, J. I., *Proteomics* 2009, *9*, 1683–1695.
- [40] Xu, D., Suenaga, N., Edelmann, M. J., Fridman, R., Muschel, R. J., Kessler, B. M., *Mol. Cell. Proteomics* 2008, 7, 2215–2228.
- [41] Cheng, F.-y., Blackburn, K., Lin, Y.-m., Goshe, M. B., Williamson, J. D., *J. Proteome Res.* 2009, *8*, 82–93.
- [42] Gnjatic, S., Nishikawa, H., Jungbluth, A. A., Güre, A. O., Ritter, G., Jäger, E., Knuth, A., Chen, Y.-T., Old, L. J., *Adv. Cancer Res.* 2006, *95*, 1–30.
- [43] Chen, Y., Scanlan, M. J., Sahin, U., Türeci, Ö., Gure, A. O., Tsang, S., Williamson, B., Stockert, E., Pfreundschuh, M., Old, L. J., *Proc. Natl. Acad. Sci. USA* 1997, *94*, 1914–1918.
- [44] Kurashige, T., Noguchi, Y., Saika, T., Ono, T., Nagata, Y., Jungbluth, A., Ritter, G., Chen, Y.-T., Stockert, E., Tsushima, T., Kumon, H., Old, L. J., Nakayama, E., *Cancer Res.* 2001, *61*, 4671–4674.
- [45] Murphy, R., Green, S., Ritter, G., Cohen, L., Ryan, D., Woods, W., Rubira, M., Cebon, J., Davis, I. D.,

Sjolander, A., Kypridis, A., Kalnins, H., McNamara, M., Moloney, M. B., Ackland, J., Cartwright, G., Rood, J., Dumsday, G., Healey, K., Maher, D., Maraskovsky, E., Chen, Y., Hoffman, E. W., Old, L. J., Scott, A. M., *Prep. Biochem. Biotechnol.* 2005, *35*, 119–134.

- [46] Silva, J. C., Denny, R., Dorschel, C. A., Gorenstein, M., Kass, I. J., Li, G.-Z., McKenna, T., Nold, M. J., Richardson, K., Young, P., Geromanos, S., *Anal. Chem.* 2005, 77, 2187–2200.
- [47] Silva, J. C., Gorenstein, M. V., Li, G.-Z., Vissers, J. P. C., Geromanos, S. J., *Mol. Cell. Proteomics* 2006, *5*, 144–156.
- [48] Gilar, M., Olivova, P., Daly, A. E., Gebler, J. C., Anal. Chem. 2005, 77, 6426–6434.

- [49] Millea, K. M., Kass, I. J., Cohen, S. A., Krull, I. S., Gebler, J. C., Bergerb, S. J., *J. Chromatogr. A* 2005, *1079*, 287–298.
- [50] Gilar, M., Olivova, P., Chakraborty, A. B., Jaworski, A., Geromanos, S. J., Gebler, J. C., *Electrophoresis* 2009, 30, 1157–1167.
- [51] Gilar, M., Olivova, P., Daly, A. E., Gebler, J. C., J. Sep. Sci. 2005, 28, 1694–1703.
- [52] Patel, V. J., Thalassinos, K., Slade, S. E., Connolly, J. B., Murrell, A. C. J. C., Scrivens, J. H., *J. Proteome Res.* 2009, *8*, 3752–3759.
- [53] Li, C., Zhang, Y.-M., *Heredity* 2010, in press. DOI: 10.1038/hdy.2010.97.